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SEASONAL VARIATIONS IN SOME PROPERTIES OF ARABLE SOIL

I. VARIATIONS IN OAT PLANT-PRODUCING CAPACITY

by G. RAPPE

INTRODUCTION

The author [8] [10] has studied the seasonal variations in plant growth in pastures and in connection with these studies investigations have been made in order to find out whether a corresponding seasonal variation could be observed in the biological properties of the soil itself. One investigation was made in 1929–30 with soils from Southern Sweden, another in 1943–45 with soils from Southern Central Sweden and from North Sweden. Only limited portions of the results have so far been published [6] [7] [9] but it is the intention of the author to publish them in full in a series of papers of which this is the first. The data in this paper are from the investigation of 1943–45, which was carried out at the Royal Agricultural College of Sweden at Ultuna and at the Swedish Grassland and Peat Cultivation Society of which latter the author was then head.

Now that the first part of the results of this investigation is being published the author wishes to offer his most sincere thanks to all those who have assisted in the investigation. Especially to my co-workers at the Grassland an Peat Society, Messrs. H. Hjertstedt, W. Heinemann, and G. Frycklund who have been responsible for various technical parts of the work. Assistants, Messrs. V. Karlsson and O. Jansson, Misses B. Almqvist, E. Gustafsson and E. Ekwo have also assisted in the work. A number of scientists at other institutions who have helped in various ways and whom I wish to thank are Professors H. Lundegårdh, S. Mattson, H. Egnér and H. Burström, Dr O. Tedin and Messrs. I. Ekdahl, G. Köhler and N. Karlsson. The experiments at the various sites have been in the care of Messrs. J. Andersson, T. Bernström, H. Liljeblad, V. Larsson, E. Thorsén and G Gustafsson.

1. SOILS AND SOIL SAMPLING

The experiment was carried out with a well-drained moraine soil rich in humus and containing some clay (soil A), a poorly drained lowmoor soil (B), a well-drained Sphagnum soil (C), a well-drained, sandy silt soil rather rich in humus (D), and a well-drained lowmoor soil (E). Of these, A, B and C were moderately well cultivated soils situated in a region northwest of Uppsala (southern central Sweden) whereas D and E were well-cultivated soils of two state experimental farms in the far north of Sweden.

When air-dried, the soils had the following weights per litre A: 1150 g, B: 260 g, C: 180 g, D: 1165 g, and E: 220 g., and on ignition the following losses were observed expressed as % age of dry material. A: 7, B: 74, C:94, D: 5, and E: 83.

Sampling areas, each of 60 square metres, were marked out on soils A, B and C at the beginning of April and areas, each of 30 square metres, on soils D and E at the beginning of May, 1943. The areas were fertilized at the rate of 40 kg of P_2O_5, as superphosphate and 80 kg of K_2O as 40% potassium chlorid per hectare; in addition ground limestone at the rate of 2000 kg per hectare in the case of soils A, B, D and E and 3000 kg per hectare in the case of soil C. The soil of each area was then carefully dug to a depth of 20 cm turned over twice and collected into a cone-shaped heap which was turned several times, the soil thus being thoroughly mixed with the fertilizers and the lime. This mixture was then spread over the original area from which the soil was derived. No further fertilizer or lime were added during the experimental period (2 years). In the summer following this treatment the average reactions of the soils expressed in pH units were: A: 5.7, B: 6,1, C: 4.4, D: 6,1 and E: 5.5.

On soils A, B, and C the sampling areas were divided into four equal plots. In each case one plot, designated A II, B II and C II respectively, was kept free from vegetation throughout the entire experiment and was weeded every time samples were taken. On May 3, 1943 the other three plots of each area were sown with oats (at the rate of 250 kg per hectare) and with a mixture of clover and timothy grass seed (at the rate of 26 kg per hectare) in such proportions as are ordinarily required in those regions for obtaining normal hay production on the types of soil in question. The original plan of the experiment had to be modified, and only one of the three cropped plots from each sampling area was sampled in the first year, another of them in the second year, both in turn being designated A III, B III and C III, respectively. Each of the six plots sampled was divided into one main section of 10 square metres from which the samples were taken and one reserve section of 5 square metres from which at each sampling a sufficient amount of soil was taken to fill up the sampling holes.

On all the cropped areas the oats were harvested on July 20, 1943. The

remaining clover-grass mixture, which was then covering the areas, was left untouched in 1943 but was cut and sampled every 14 days in 1944. Only one half of each plot was cut and sampled on every sampling date, the result being that each half of the plot was sampled and harvested every 28 days.

At the beginning of the experiment a certain amount of soil was taken from one of the unused plots of soils A, B and C and was spread in a 40 cm layer on the floor of an ordinary potato cellar where the air temperature varied during the whole year from 7° to 11°C. These cellar soils were designated "KA", "KB" and "KC" respectively, and sampling from these commenced $1^1/_2$ months after spreading on the cellar floor.

The entire sampling areas of soils D and E were at the end of May sown with oats and a mixture of clover and grass in the same way as soils A III, B III and C III. One half of each area was used for sampling in the first year of the experiment and the other half in the second year of the experiment. In each of these halves 5 square metres were resolved for filling up the sampling holes made in the remaining 10 square metres of the area. Harvesting and sampling were carried out in the same way as described above.

Sampling of soils II (A, B and C) commenced on April 15, 1943, on soils III on May 27, 1943, on soils D and E on May 12, 1943, and on soils K (A, B and C) on June 25, 1943. On all the A soils the sampling was carried on until April 26, 1945 while on the other soils it was discontinued on Oct. 25, 1944. The soil samples were taken so as to arrive in the laboratory on the same day as they were taken. All the samples arriving on one day are referred to as a "set", the numbers of these are found at the bottom of figures 5–8.

On each occasion samples of 30 litres of each field soil were taken so as to include the whole profile, down to a depth of 20 cm. The sampling holes were immediately filled with soil from the reserve areas; on cropped soils the sward, too, was restored, fresh plants being supplied from the said reserve areas. — At the same time samples of 1 litre were taken in a similar manner from each of the soils (KA, KB, and KC) stored on the floor of the potato cellar.

During that part of the winter when the field soils were frozen one simple sample was taken with the aid of a pick-axe. Filling up the holes and "mending" the sward was postponed until the soils had thawed in spring.

Immediately upon their arrival at the laboratory the samples were sifted through 10 mm sieves and thoroughly mixed. Frozen or highly water-saturated soils were first spread out on wire mesh for thawing and draining away of surplus water; if necessary they were subjected to a gentle drying treatment with the aid of a fan. After sifting the samples obtained from the 11 soils, were each divided into several portions to be subjected to various special studies, one of which will be described here. Details regarding the treatment of the soils in these studies and the methods employed are given in a separate report by the author and H. Hjertstedt*).

*) Copies of this report may be ordered from the author directly [11]).

2. METHOD OF DETERMINING THE CAPACITY OF THE SOILS TO PRODUCE PLANT MATERIAL

The method employed for determining the capacity of the soils to produce plant material and studying seasonal variations in this capacity is based on the following principle: a certain number of oat plants are grown for a certain number of days on a quantity of soil from each sample which corresponds to a fixed quantity of dry matter for each soil, and the amount of dry matter produced by the oat plants determined. Oats were considered suitable because of their relative insensitiveness; compare E. A. Mitscherlich, 1925 [5]). The oat seed used has been of uniform quality throughout the experiment, and all the samples have been given as nearly as possible identical treatment with regard to temperature, light, irrigation etc. The author was able to fulfill the latter condition due to the kindness of Prof. H. Lundegårdh, who permitted him to use the photo-thermostat of his Institute during two years.

This photothermostat was placed in a dark cellar with 60 cm concrete walls and consisted of two chambers having a length of 90 cm, a depth of 50 cm and a height of 80 cm. A few cm above the bottom a horizontal plate carried the experimental pots. In the centre of the bottom there was an opening (30 mm in diameter) connected to a tube for letting in air from the immediate surroundings of the building. Air circulation within each chamber was provided by a Siemens table fan. The "roof" of the chamber consisted of two horizontal glass plates; in the 10 cm space between the two plates a stream of water was kept constantly circulating. Each chamber was illuminated from above the glass plates by two electric bulbs of 1.000 watt each. Throughout the experiment the lamps were kept burning for 16 hours per day, *viz.* from 5 p.m. to 9 a.m. In the remaining 8 hours of the day the photothermostat was kept dark. During the illumination period temperatures varied from 19 to 22°C and during the dark period from 14.5 to 15.5°C.

From each sample in its natural condition of moisture two portions of soil were taken so that they contained the same amount of dry matter which was kept constant throughout the experiment for all samples of one and the same soil. Each portion was placed in an ordinary flowerpot. The quantities of dry matter per flowerpot were as follows: soils A and D: 534 g, soils B and E: 107 g, and soil C: 84 g. Thus, at each sampling 22 flowerpots were filled with the quantities of soil indicated above and sown with oats, the seed always coming from the same lot of "Guldregnshavre II" from the 1942 crop, and retained a high germination capacity throughout the experiment. 15 seeds were sown in each flowerpot, but after germination the number of plants was reduced to 10 per flowerpot.

The seeded pots were immediately installed in the photothermostat where they remained for 35 days. Water was supplied twice a day, the flowerpots being weighed to ensure addition of sufficient water to maintain the water content of the soils at about 60% of their maximum capacity. After each irrigation the pots were rearranged within the thermostat, the purpose being to eliminate any possible differences in the environmental conditions in different positions.

In view of the frequent watering the humidity in the chambers was assumed to be so high that any fluctuations in humidity caused by air coming in from the outside could be regarded as negligible.

35 days after sowing the oat plants were cut off close to the soil surface. It was not possible to treat the yields from two parallel flowerpots separately, and these yields were therefore first weighed separately and then airdried together in an open box, and weighed again. The data obtained from these weighings are graphically represented in Figure 1*a*. Tables of the data for Figures 1*a*, 2 and 3*a* cannot be published for lack of space but are available upon application to the author. The aerial parts of the plants were analysed spectographically, the results will be published in subsequent papers.

Immediately after each harvesting of the aerial parts of the plants, the oat roots were rinsed and air-dried and weighed in the same way as the tops. The weight data obtained are given graphically in Figure 2. Some difficulties arose in attempts to completely free the root systems from soil, particularly in the case of the lowmoor soils but also in the Sphagnum and mineral soils. It is assumed, however, that the error involved was approximately constant for each soil throughout the experiment. Thus although the weight data may fail to give the true root weight it is hoped they represent with sufficient accuracy the seasonal variations in the weight of the root mass.

The photothermostat in which the experiment was carried out had been used for several years for growing various cereals, mostly wheat in nutrient solutions. Professor H. L u n d e g å r d h confirms that plant growth in this photothermostat was uniform during the whole year, irrespective of the season. This statement agrees well with the author's own observation as to the germination rate of the oats, which appeared to be quite uniform throughout the year. It was therefore assumed that the quantities of oat material produced at different seasons could be taken as a direct measure of the variations

in the status of the soils studied. Whether or not this assumption is correct will be shown to some extent by investigations being carried out during the present season. This matter has taken on a somewhat different aspect because of the findings of E. Bünning [2] [3] and it is the writer's intention to make germination and early development of oats under constant conditions the subject of special study as a supplement to the present series.

3. EXPERIMENTAL RESULTS

Due to the large amount of experimental material it was impossible to determine the amount of dry matter for each sample, and I have been obliged to use the values for air dry weight instead. Drying has been carried out under uniform conditions (the apparatus used is described by Lundegårdh [4]) and it may be safely assumed that the moisture content of the hay has been constant throughout the experiment. As mentioned above, the data for root weight are not absolutely exact due to difficulties in complete removal of soil but they are never-the-less considered to reflect well enough the seasonal variations studied in these experiments.

In all the Figures, continuous vertical lines mark summer and winter solstices whereas broken vertical lines mark vernal and autumnal equinoxes. In Figs. 1*a*, 2, 3*a* and 4 the dates of solstices and equinoxes as well as of the first and the last samplings are given at the bottom of the diagram. A so-called "weather strip" is given at the top of Figs. 1*a*, 2 and 3*a*, consisting of a series of letters indicating the general character of the weather prevailing during the period covered. The letters of the weather strips have the following meaning:

V = very warm
v = normal warmth
k = somewhat subnormal temperature
K = markedly subnormal temperature
} estimated from weather diagrams already published by the author

T = very dry, less than 5 mm precipitation per week
t = dry, 5–10 mm precipitation per week
f = wet, 10–20 mm precipitation per week
F = very wet, more than 20 mm precipitation per week.

The complete weather diagrams on which the above strips are based have been published by the author [8] [10].

Figs. 1*a* and 2 show the variations in weight of tops and roots, respectively. The graphs of these Figures, as well as those of Figs. 3*a* and 4, have been constructed from the detailed data from each sampling or on calculations made therefrom. Smooth curves have been constructed from the original graphs in the following way; the primary value for each date in the original graph was replaced by the mean of this value and those immediately preceding and

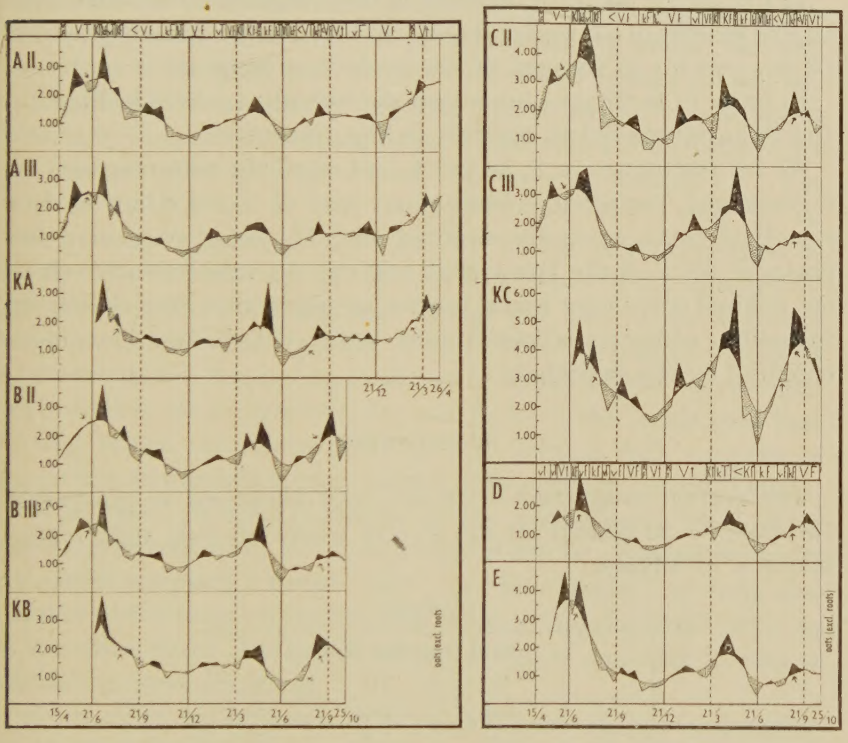

Fig. 1*a*. Variation in the air dry weight of oat tops. (20 oat plants grown for 35 days on samples of soils A–E in a photothermostat, fresh samples being collected every other week). First sampling date April 15, 1943. Weight in grams from 20 plants grown in two pots.

following it. The primary values of the original curve have then been replaced by secondary values, each being mean of three primary values. The curve thus obtained was subjected to the same treatment, and this procedure was then repeated twice more. The final curve (shown in the diagrams together with the original curve) thus

being the product of a fourfold smoothing process. Wherever the smoothed curve lies above the original one the space between the two has been dotted and where the smoothed curve lies below the original one the space between the two has been blocked in. Of course only the central portions of the curves, viz. from the fifth point from the beginning to the fifth point from the end, could be smoothed out fully; these points have been marked by arrows.

In Fig. 3*a* the points of the original curve represent the sum of the air dry weights of oat tops and roots; in Fig. 4 they represent the root air dry weights in per cent of the air dry top weights.

In order to facilitate comparison the smoothed curves in Figs. 1*a* and 3*a* have been drawn together in Figs. 1*b* and 3*b*.

As for the figures 1*a*, 2, 3*a* and 4, and especially as far as Fig. 1*a* is concerned, periodicity observations may be based either on the smoothed curve or on the original one. This is of particular importance since, if the two curves are viewed against each other, the original curve may reveal certain regularly recurring details in the course of the variations studied, which details may have been obscured by the smoothing.

4. OAT TOPS

The average production of air dry tops expressed in grams per two pots (= 20 plants) for the 36 sets where all soils were represented is as follows:

TABLE I

Soil	Type of treatment of soil:		
	II	III	K
A	1.24	1.08	1.44
B	1.45	1.29	1.59
C	1.81	1.67	2.96
D		1.08	
E		1.33	

The analysis of variance discussed in section 9 gives a standard error of 0.10 for a difference between two soil-means from these 36 sets. Soil B is thus fairly significantly more productive than soil A, soil C is clearly superior to both A and B. Soil D is about equal to A and soil E to B. Soil stored in the cellar is very significantly more productive than the field soils (II, III) in the case of soil C, and its

superiority is strongly indicated also in the case of soils A and B. The difference between bare and cropped field soils (II and III) are discussed at length in section 4*b* below. The high productivity of the cellar soils may, at least in part, be explained by the fact that these soils showed greatly increased microbiological activity which have rendered them particularly rich in nutrients.

From Figures 1*a* and 1*b* it will be observed immediately that on most soils oat plant production was much greater during the spring and early summer than at other periods of the expriment, the only exception being in the case of the cellar-stored Sphagnum soil KC.

The increased productive capacity appearing during the spring and early summer is associated with initial activation (Romell) a phenomenon discussed previously by several workers *e.g.* Th. Schloesing[20]), L. G. Romell[16]) [17]) [18]) [19]), and S. A. Waksman and R. L. Starkey[21]). This activation must be ascribed to the treatment of the soils at the start of the experiment, primarily to the manuring and liming, but also to the thorough mixing which was not repeated. Schloesing studied this effect as it appeared in fermenting snuff and he found it to be unconnected with differences in aeration. He put forward the hypothesis that mixing brings the microorganisms into contact with supplies of previously untouched organic matter, this results in increased microbial activity. Romell looks at the phenomenon from more general points of view. He explains that it can be expected to result, *e.g.*, from sampling a forest "mor", or "raw humus", or from disruption of the connections between the mor and the forest trees, as in trenched plots. The whole effect is viewed as being in part a parallel to green manuring.

Romell's hypothesis may be taken as a convenient explanation for the fact that the initial activation appears with greater intensity in the heighly organic soils C and E which have a rather well-preserved structure than in the organic soil B in which the plant components are more completely disintegrated, — and also with greater intensity in all the organic soils (B, C and E) than in the mineral soils (A and D).

It seems that the effect of this initial activation has been overcome in all the experimental soils by the end of the summer of 1943 which thus marks the beginning of the period in which all the experimental data may be assumed to be comparable among themselves

and reliable enough to reveal any variations due to seasonal influences. The end of this period was late in the autumn of 1944 when sampling was discontinued on all the soils except the A soils. This period will hereafter be called the "main experimental period" while the time of initial activation will be called the "preparation period". The experimental work carried out after the "main period", with the A soils only, will be referred to as the "continued A experiment". It is true that during the "main experimental period", too, the soils examined have undergone considerable disturbance due to sampling, mixing and sifting which must have resulted in abnormal microbial activity. This disturbance was, however, very similar at every sampling for, as long as the soils were not frozen, this procedure was always exactly the same. It is therefore assumed that this procedure did not cause any changes which would be confused with seasonal changes in the soil properties examined. The *uniform* changes it may have caused probably lie in the direction of higher production values than those which would have been obtained if it had been possible to carry out the experiment without transporting the soils from their natural position into a photothermostat and without all the necessary manipulations of the experimental technique.

a. *Rhythmic features common to all the soils examined*

A rhythmical, seasonal variation in productive capacity more or less pronounced in all the 11 soils will be readily observed from the diagrams. This rhythm appears most clearly in the three Sphagnum soils (C), and especially in the KC soil. The lowest values in all the curves are those of the summer and winter of the "main experiment period" and the highest values are those of the late spring and early autumn of the same period. In the "continued A experiment" oat plant production did not decrease as much in the winter of 1944–45 as it did in the winter of 1943–44, but a downward tendency is nevertheless visible. The high values observed in the spring of 1944 recur undiminished in the spring of 1945.

Thus, during the "main experiment period" as well as in the "continued A experiment" there clearly occurs a *regular, seasonally controlled rhythm comprising high values in late spring and early autumn and low values in summer and winter*. Furthermore, it seems that the low values of the winter season extend over a longer period than those of the summer season; the high value periods of both

spring and autumn are therefore closer to the summer period.

However, a close examination of the curves shows a further group of relatively high values in the neighbourhood of the low winter values.

Reference is made in section 9 to the statistical significance of these "secondary" maxima and minima.

As a result of these secondary maxima in the winter half of the year, there occur two further periods of comparatively low values between the high-value periods after and before the summer periods of lower productivity. The low-value periods of autumn, winter and spring have their minimum just before the autumnal equinox, the winter solstice and the spring equinox respectively. The low values occurring in summer are centered exactly around the summer solstice.

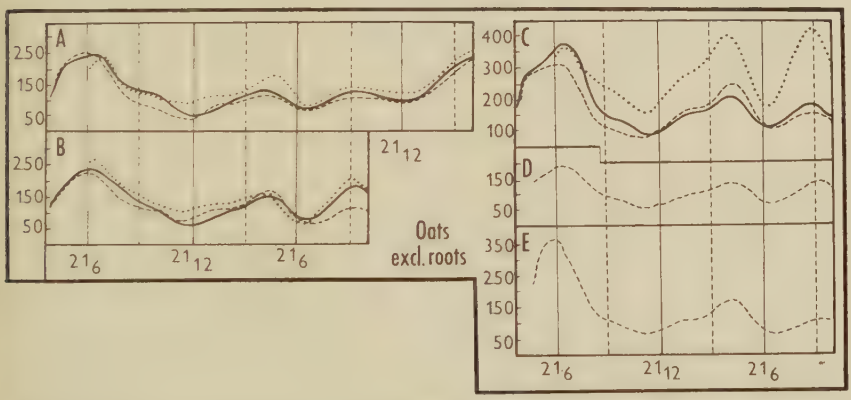

Fig. 1*b*. The curves of Fig. 1*a* after smoothing: — bare soils, – – – cropped soils, ... cellar soils.

Since, however, the periods of low production are much less pronounced in autumn and spring than in winter and summer it seems adequate to describe the general trend of the curves in the following way: There are two strongly marked periods of low productive capacity, one around midsummer and one some weeks before midwinter, while in spring and autumn there occur periods of high production nearer to the minimum value of the summer than to that of the winter, these high production periods again being interrupted by secondary minima just before the equinoxes.

The rhythmical, seasonally controlled variations described above

are clearly visible in the curves for all the 11 soils studied. They are, however, more strongly marked in some soils than in others. These differences in the strength of the seasonal variations have been studied by a comparison of the inter-set variations for different soils (cf. section 9, page 217). The differences in inter-set variations, as given on page 218, coincide rather well with the differences in seasonal amplitudes as shown by Figure 1*a*. It seems, justified therefore, to use the data presented in section 9 to estimate differences between the soils regarding the amplitude of the seasonal variations.

It is then evident, that the seasonal variations are much more pronounced in the Sphagnum soil (C) than in the mineral soils (A and D) and low-moor soil (B). Soil E is more or less intermediate between B and C, which agrees well with its intermediate nature, as is demonstrated by data given in the first paragraphs of section 1. In the case of soil C the seasonal variations seem to be especially strongly marked in the cellar soil (KC); similar differences are not indicated in soils A and B.

b. *Comparison between the results obtained for cropped and for bare field soils*

In Figure 1*b* the curves of both cropped and bare field soils are shown, side by side for soils A, B and C. From this figure it appears clear that while the two curves for each soil both follow the general rhyhtm described under (*a*) they do not run parallel.

Middle and late summer as well as autumn cropped soils show lower oat plant-production capacity than bare soils; the explanation may be that cropped soils are repeatedly impoverished in nutrients by growing plants. In winter, spring and early summer, the curves for the cropped soils rise above those for the bare soils; during these seasons the roots and stubble remaining in the cropped soils from the preceding autumn may perhaps provide extra organic material, for the activity of microorganisms, which is lacking in the bare soils.

The above difference between cropped and bare soils appears most clearly in the case of soil C, less clearly in the case of soil B and still less pronounced in the case of soil A. Apparently organic and especially Sphagnum soils are more suitable for comparative investigations of this kind than are mineral soils.

5. OAT ROOTS

As mentioned in section 2 the determination of root weights met with considerable difficulties and comparisons between soils regarding root productivity have, therefore, been considered as more or less valueless.

Also the comparisons between soils concerning the inter-set variability, as made in regard to yield of tops, are very uncertain. There hardly seems to be any reason for a more detailed discussion of the data given in section 9 in this respect.

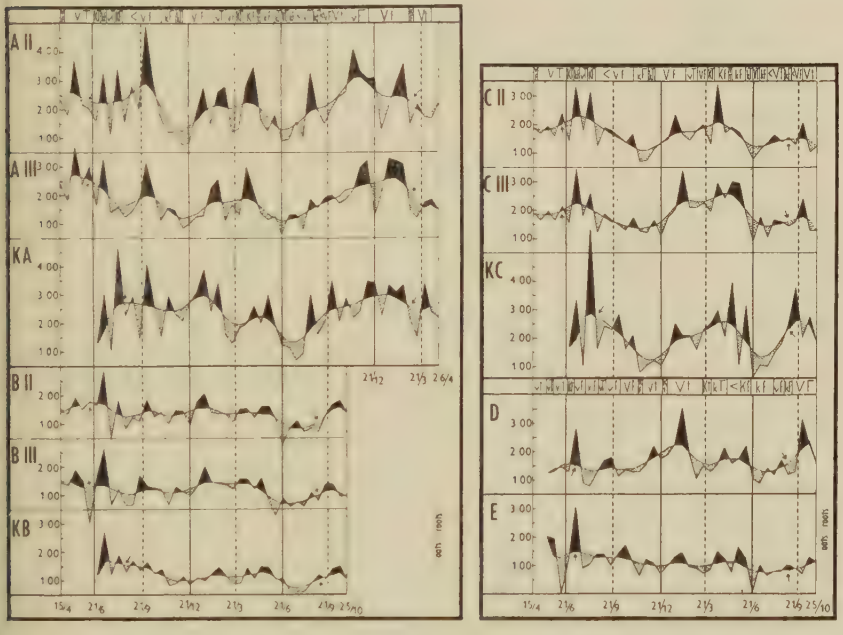

Fig. 2. Variation in the air dry weight of the roots of the same oat plants as in Fig. 1*a*. Weight in grams from 20 plants grown in two pots.

In spite of the irregularities in the curves for the roots the same seasonal rhythm as in the curves for the tops can be observed but with minor differences.

The summer period of low production is less pronounced and shows a tendency to occur somewhat later than in the curves for the tops, while the winter period of low production, which is also weaker than in the curves for the tops, tends to occur a little earlier. The two

secondary low production periods of autumn and spring appear more clearly in the roots than in the tops, especially in the case of the

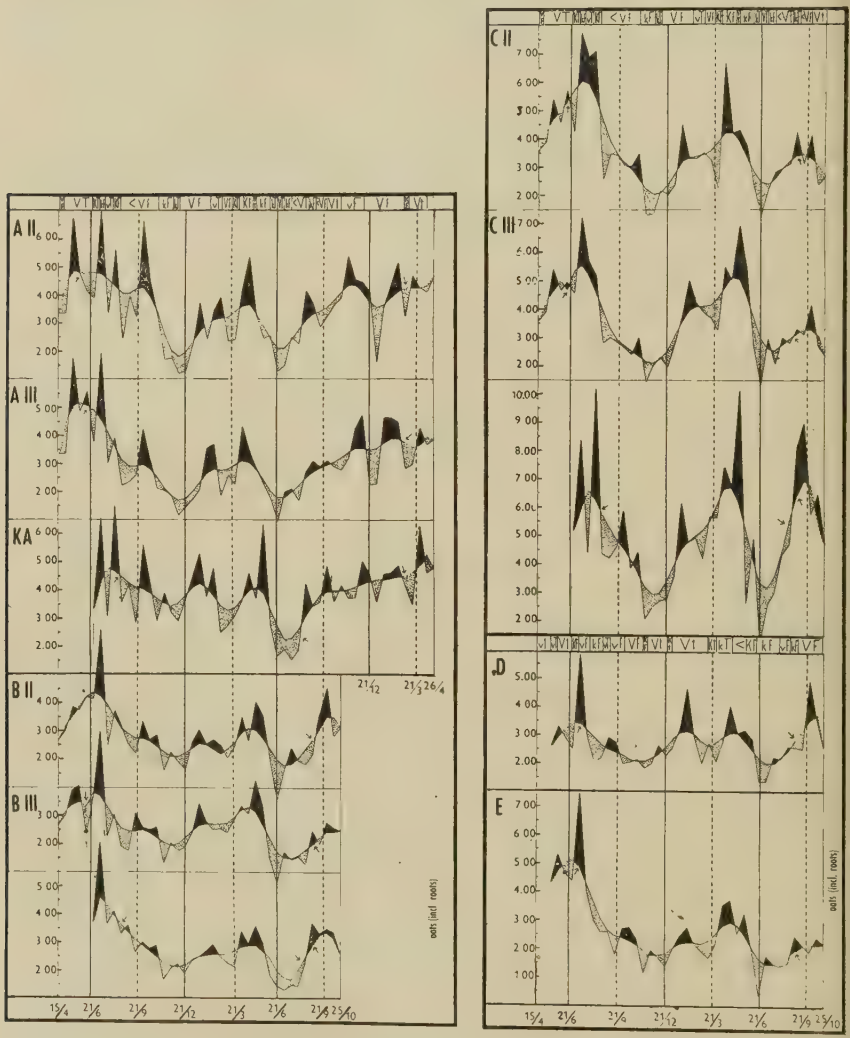

Fig. 3*a*. Variation in the air dry weight of tops + roots of the same oat plants as in Fig. 1*a*. Weight in grams from 20 plants grown in two pots.

two mineral soils A and D in which root weight determinations were probably more reliable than in the other soils.

The high initial valies, resulting from the activation effects, dis-

cussed in section 4 are far less pronounced in the curves for the roots than in the case of the curves for the tops. This would be expected if the variations were due to differences in the level of available nitrogen (cf., *e.g.*, the several papers of M. E. Reid [12] [13] [14] [15]).

6. OAT TOPS + ROOTS

The curves in Figures 3*a* and 3*b* represent the sums of top + root weights. The difficulties pertaining to the harvest of roots must of course influence the sums of tops + roots. No comparisons between soils have been made in respect of total productivity or to inter-set variations for tops + roots. On the other hand, the seasonal rhythm discussed in sections 4 and 5 for tops and roots separately will also be studied from the curves representing the total production of plant material i.e. tops + roots.

a. *Rhythmic features common to all the soils examined*

The rhythm appearing in the curves of Figs. 1*a*, 1*b*, and 2 is even more striking in the curves of Figs. 3*a* and 3*b*, the difference between

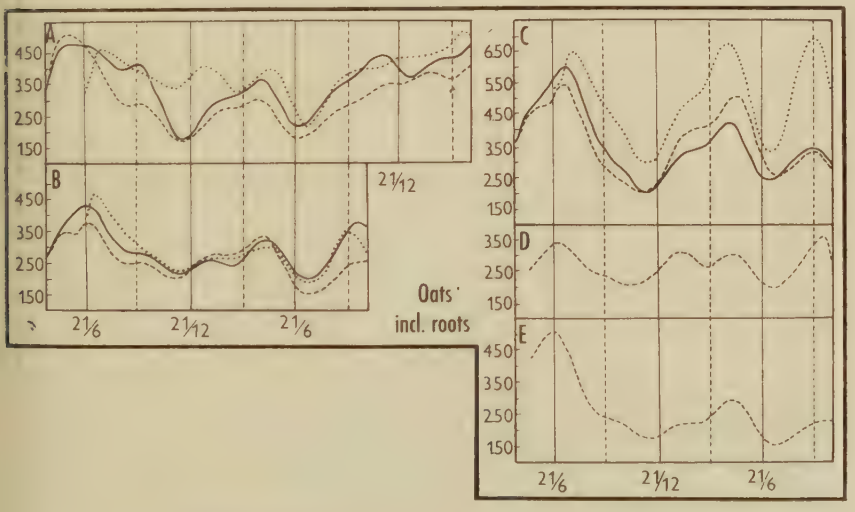

Fig. 3*b*. The curves of Fig. 3*a* after smoothing.
Bare soils, – – – – cropped soils, ... cellar soils.

the highest and the lowest values being greater in the original as well as in the smoothed curves. In comparison with the curves for the

oat tops the top + root curves show a more marked low production period in the winter than in the summer and the lowest summer values are reached a couple of weeks later than in the curves for the tops. The low production period in spring is more marked in the top + root curves than it is in the curves for the tops but occurs at the same time in both. The curves of Figs. 3*a* and 3*b* thus give a stronger impression of four modal points than those of Figs. 1*a* and 1*b*, although the unsmoothed curves of Figs. 1*a* and 1*b* are more regular.

b. *Comparison of the results obtained for cropped and bare field soils*

The difference in productive capacity between cropped and bare soils observed in the case of oat tops material also comes out when the tops and roots are taken together (Fig. 3*b*), and is very marked in the B and C soil groups. In the A group the curves for the winter values for the two soils run close to each other whilst the curve for the bare soil values rises far above that for the cropped soil for the remaining seasons, especially the autumn.

7. WEIGHT OF OAT ROOTS EXPRESSED AS A PERCENTAGE OF OAT TOPS

The curves of Fig. 4 have been constructed in exactly the same way as those of Figs. 1*a*, 2 and 3*a*.

There do not appear to be any regularly occurring or seasonally controlled differences between the curves of the bare and the cropped soils, but a seasonal rhythm more or less common to all the soils is readily apparent. For most of the soils root percentages are particularly high in winter, total oat plant-production as well as oat top production being comparatively low. Around midsummer another period of low production occurs but at this time root percentages are not so high as in winter except in the KB and KC soils which have their highest root percentages at midsummer. In the activation period at the beginning of the experiment there is no indication of a midsummer maximum in root percentage.

8. MEAN CURVES FOR ALL SOILS

It has been possible to demonstrate a seasonal rhythm concerning production of tops, roots, tops + roots and also for the ratio roots:

tops for all the 11 soils. It has been considered advisable to calculate the means for all soils for each set and to present the values thus

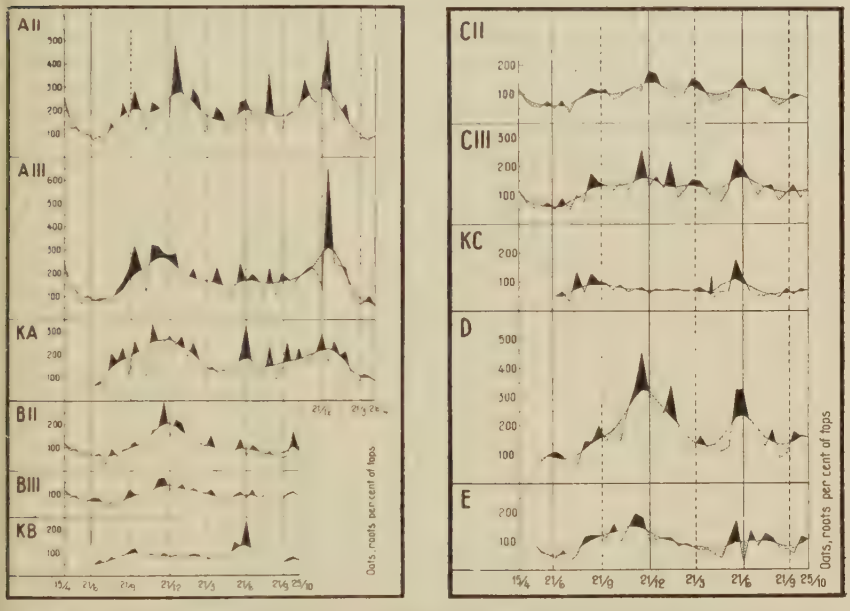

Fig. 4. Variation in the air dry weight of the roots of the same oat plants as in Fig. 1*a*, expressed as a percentage of the air dry weight of the corresponding tops.

obtained graphically. The curves of the means are given in Figures 5 (tops), 6 (roots), 7 (tops + roots) and 8 (roots as per cent of tops). Some statistical analyses of the data for these curves are presented in section 9; in this section a comparison is made between the four curves in order to give a comprehensive survey of the observations discussed in sections 4–7.

Figures 5–8 are all similarly constructed. In the centre of the figure is a mean curve for all 11 soils, including the whole of "the main experiment period" (sets 10–41) and the last sets (6–9) of "the preparation period". On the lower left, is a curve of means for the six field soils from Southern Central Sweden. The continuous line of this curve includes the first sets (1–5) when not all the soils were sampled, the broken line indicates the mean curve for these six soils up to the vernal equinox in 1944 (sets 6–25). Finally the curve on the upper right represents the means for the three mineral soils from Central Sweden which were sampled until the late spring of 1945. The continuous line of this curve includes "the continued A

experiment period" (sets 42–54), the broken line the latter part of "the main experiment period", from the winter solstice in 1943 onwards (sets 19–41).

The vertical lines with dates at the base-line indicate, as in other figures, the summer and winter solstices and the vernal and autumnal equinoxes. Along the base-line of each figure the number of each set included in the curve is printed. Finally the ordinate figures indicate weight in grams; the values used in constructing the curves being means of 11 individual values, each comprising the sum of harvests from two pots (= 20 plants).

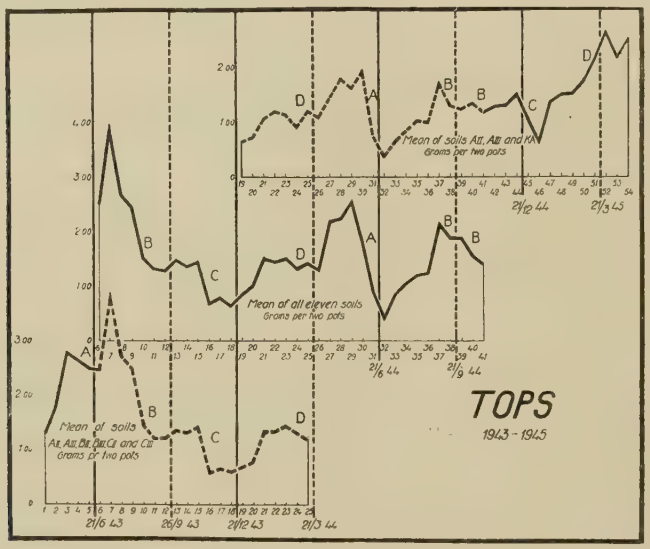
Mean of soils A0, A00 and A0
Grams per two pots
Mean of all eleven soils
Grams per two pots
Mean of soils
A0, A00, B0, B00, C0 and C00
Grams per two pots
TOPS
1943 - 1945

Fig. 5. Variation in the air dry weight of oat tops. Means of values for eleven, six and three soils, respectively.

In order to facilitate a comparison between the curves in figures 5–8 the approximate times for the beginning of downward trends resulting in the minima discussed in sections 4–7 have been marked by the letters A to D. A denotes the approximate time for the mid-summer minimum, C for the winter minimum and B and D for the secondary minima in autumn and spring, respectively.

In the following paragraphs the information obtainable from the four curves in question with reference to the four annual minima in oat plant-production and the corresponding changes in the root: top relation is discussed.

The summer minimum A is very clearly marked in 1944, both for tops, roots and their sum. Even during "the preparation period" in

1943 with its very marked activation increase in productivity there is a fairly strongly marked summer minimum. In this latter case there is no corresponding maximum in root-percentage (Fig. 8) but

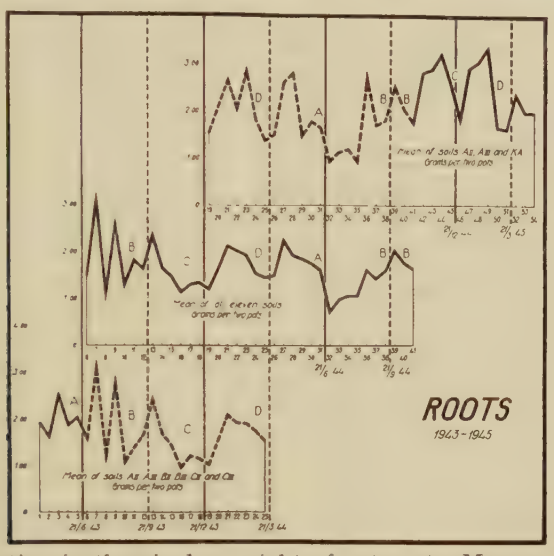

Fig. 6. Variation in the air dry weight of oat roots. Means of values for eleven, six and three soils, respectively.

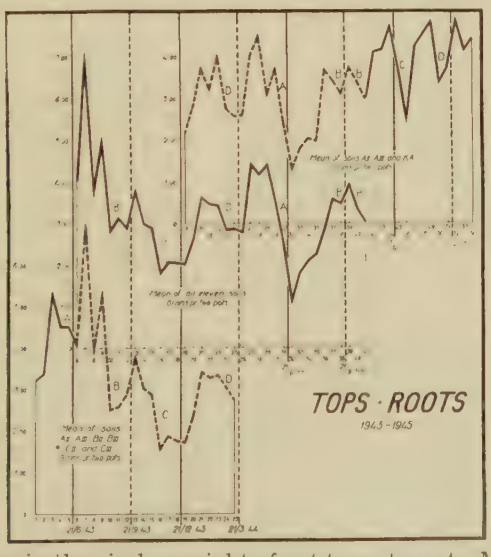

Fig. 7. Variation in the air dry weight of oat tops + roots. Means of values for eleven, six and three soils, repectively.

a marked maximum occurs at the time of the summer production minimum (A) in 1944. This difference between the two summers in regard to the root-percentage may be related to the activation discussed in section 4.

The winter minimum C is very marked in the winter of 1943–44 during the "main experiment period" as shown in all three figures 5–7 and corresponds to a marked maximum in figure 8. During "the continued A experiment" there is also a marked minimum in the winter of 1944–45, and a corresponding maximum for root-percentage. The winter minimum, however, is less marked and of shorter duration in 1944–45 than in 1943–44.

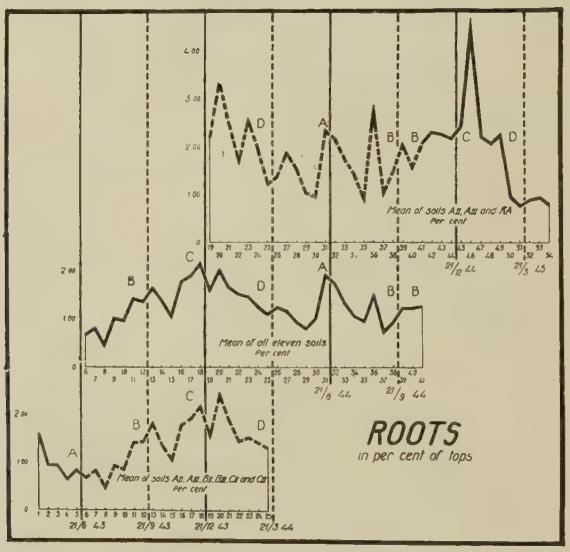
Mean of soils Aa, Aa and AA
Per cent
Mean of all eleven soils
Per cent
Mean of soils Aa, Aa, Ba, Ba, Ca and Ca
Per cent
ROOTS
in per cent of tops

Fig. 8. Variation in the air dry weight of roots as a percentage of the air dry weight of the corresponding tops. Means of values for eleven, six and three soils, respectively.

The secondary minima B and D are comparatively weak in Fig. 5 (tops). It is more a question of a break in the falling or rising trend of the curves with some indications of a real minima. In "the continued A experiment" the spring minimum (D) of 1945 is either totally absent or occurs unusually early as a break in the upward trend of the curve.

The curves for roots and for tops + roots present quite a different picture. There are marked spring minima (D) in 1944 (sets 24–26)

and 1945 (sets 50–51) and an autumn minimum (B) in 1943 (sets 10–12). In the autumn (B) of 1944 (sets 37–41) the values are somewhat irregular; this secondary minimum is somewhat more marked in the case of tops than for roots.

There are only slight indications of maxima in the root-percentage corresponding to the spring and autumn minima found for total production.

9. STATISTICAL TREATMENT OF THE DATA

The statistical calculations, referred to in some of the previous sections, have been made by Dr O. T e d i n. The methods employed have of necessity been modified to suit the nature of the data; they can only be indicated briefly here.

The variability between two parallel pots can be calculated only in regard to the harvest of green tops. An average value for the whole material has been computed after elimination of a systematical difference between the two chambers of the photothermostat observable during the first part of the experiment. This average value is 11.9% as the standard deviation of a single pot. This value decreases somewhat with increasing absolute harvests but not enough to make an absolute value preferable to the relative one.

In an open air experiment, carried out in 1951 on the same general scheme as the experiment described in this paper, but with six replicates, the relative standard deviation for (air dry) weight was on the average for eight crops 1.25 times that of the relative standard deviation for green plant material.

The values just presented have some value in giving an idea of the general reliability of individual determinations, but they are of minor importance in judging the statistical significance of some of the hypotheses of the author. Another analysis is of greater importance in this respect. In Figures 5–8 averages for all the 11 soils studied are presented. The 11 soils are not true replicates biologically, nor are they a representative sample of natural Swedish soils. Since some of the main phenomena observed occur in all 11 soils they may be used, in the absent of more satisfactory material as statistical replicates in order to obtain some estimate of the significance of some of the numerical results presented.

If the 36 sets of samples, in which all 11 soils in the experiment are represented, are used for an analysis of variance the following result is obtained:

TABLE II

Cause of variation	Degrees of freedom	Variance		
		Tops	Roots	Tops + Roots
Between soils	10	9.9380	5.7400	21.0500
Between sets	35	5.2140	2.5377	12.3800
Error	350	0.1934	0.2990	0.7139

The P-values for the inter-soil and inter-set variances due to random sampling are in all cases less than 0.001.

From the error variances the statistical significance of some phenomena may be judged. The difference between the average yields of air dry tops for two soils discussed in the beginning of section 4, has a standard error of 0.10.

In sections 4 and 5 the differences between the soils in regard to the intensity of the seasonal rhythm is discussed. The significance of these differences may be judged from the inter-set variances of these soils, or, since there are 35 degrees of freedom for each single soil, from a direct comparison between the sums of squares computed for the analysis given in the previous table. These sums of squares are as in Tables III and IV.

The quotients of interest are as follows: the signs ***, ** and * denoting P values of respectively less than 0.001, 0.001–0.01 and 0.01–0.05.

For tops: C/A = 2.94***; C/B = 2.81***; KC/CII = 1.58; KC/CIII = = 2.02*; E/BIII = 2.10*.

For roots: A/B = 3.86***; C/B = 3.29***; AII/AIII = 2.47**; KA/AIII = = 2.72**; AIII/D = 1.18; KC/CII = 2.85**; KC/CIII = 2.14*.

In sections 4*b* and 6*b* the differences between different seasons in regard to the relative productive capacity of bare and cropped soils is discussed.

TABLE III

Soil	$S(x-\bar{x})^2$	
	Tops	Roots
A II	15.30	32.80
A III	14.07	13.28
KA	13.97	36.12
A, 3 soils	43.34	82.20
B II	18.67	8.13
B III	11.53	7.44
KB	15.22	5.73
B, 3 soils	45.42	21.30

TABLE IV

Soil	$S(x-\bar{x})^2$	
	Tops	Roots
C II	37.89	13.52
C III	29.70	18.03
KC	59.90	38.58
C, 3 soils	127.49	70.13
D	9.70	11.30
E	24.22	8.79

If the two main periods in this respect are taken to extend from the winter to the summer solstice and then from the latter to the former the differences between periods as regards the differences between bare and cropped soils are:

TABLE V

Soils	A	B	C
Tops	0.21	0.31	0.55
Tops + Roots	0.32	0.45	1.02

The standard errors of these differences between differences, each computed from 4 times 13 primary values, can again be computed from the error variance in the analysis of variance by multiplying this by 4/13; these standard errors are 0.24 for tops and 0.47 for roots. None of the individual

values reaches any considerable degree of significance but together they may be considered as supporting the hypothesis put forward.

The problems so far discussed from a statistical point of view have been more or less peripheral in regard to the main hypothesis i.e. the seasonal rhythm of productive capacity observed in all the soils studied. This main hypothesis is unfortunately less susceptible to statistical analysis than any of the problems so far discussed in this section.

Each value in the "mean curves" (Figures 5–7) for the main experiment period is founded upon 11 single values. From the error variances in the analysis of variance the standard errors of these values can be computed; they are 0.13 for tops, 0.16 for roots and 0.25 for tops + roots. With the aid of these values it is possible to estimate approximately the significance of the maxima and minima of the curves.

The minima denoted by A and C seem to be quite significantly different from the maxima in spring and autumn. The minima denoted B and D are somewhat more doubtful from a statistical point of view. The differences between consecutive points around these minima are of about the same order as the standard error, a minimum represented by a single point can therefore hardly be ascribed any significance. When a minimum is represented by several consecutive points its significance obviously increases, but it becomes mainly a question of judgment by common sense if one wants to consider their existence as proven.

DISCUSSION

The results of the experiment reveal a marked seasonal rhythm in the oat-plant-producing capacity of soil samples assayed in the phototherostat. It seems quite remarkable that the amplitude of the variations observed is not less in the cellar soils than in the field soils; within the C soil group it is even considerably greater. The amplitudes of the curves from North Swedish soils may be said to cover about the same range as those of the curves from southern central Swedish soils.

The seasonal variations common to all the soils examined can hardly be ascribed to meteorological influence, for if this were the case they would naturally be less marked in the cellar soils which, however, is not the case. Nor can they be ascribed to such variations in environment as will result from different cultivation or different geographical position, for in that case different rhythms would be exhibited for bare field soils on one hand and for cropped field soils on the other, in addition North Swedish soils would behave differently from southern central Swedish soils on the other, neither of which is the case.

Even before the beginning of the experiment the author was quite prepared to find a decline in oat-plant-production some time in the middle of the summer since on many occasions an analogous phenomenon had been observed in pastures. Around midsummer, pasture grasses appeared to recover from grazing less easily than during other periods of the growing season. The object of the photothermostat experiment was therefore to investigate whether the soils actually passed through a stage of reduced productive capacity which would correspond to the reduced growth rate of the grasses around midsummer. In the first place this was undertaken regardless of any considerations of variations in nutrient materials or other factors within the soils themselves that might explain the occurrence of such a phase of reduced plant growth. The effect of variations in these individual soil factors have also been examined, and the results will be published in future papers.

SUMMARY

The experiment was carried out with five different soils, viz. two mineral, two low-moor and one Sphagnum soil.

A given area of all of these soils was thoroughly dug and turned over; in three of the soils one half of the area and in two of them the entire area was sown with oats and a mixture of grass and clover. The other half of the area in the three soils was kept free from vegetation. In addition a layer of these soils 40 cm in thickness was spread out on a cellar floor.

Samples were taken every other week from all the 11 soils and the soil of each sample divided between two flowerpots which were then immediately sown with oats and put into a photothermostat where they were left for 35 days. After 35 days, the oats were cut off level with the soil surface, air-dried and weighed. Soil was removed from the roots of the oatplants which were then also air-dried and weighed.

Figures 1–4 represent curves prepared from the experimental data: (i) curves founded directly upon the values obtained in the course of the experiment and (ii) curves constructed by means of a fourfold smoothing procedure.

The curves of Figs. 1–4 reveal clearly a general seasonal rhythm common to all the 11 soils studied in the photothermostat: there is a considerable decline in the growth of oat plants in the middle of the summer and somewhat before the middle of the winter, and a less marked decline just before the vernal and autumnal equinoxes.

Further, the curve for the growth of oat plants on the cropped soil falls below the curve for growth on the bare soil in the middle of the summer and also in autumn but rises above it in winter, spring and early summer.

The very marked decline in the plant production capacity occurring in the middle of the summer agrees well with the author's observations made on ordinary pastures where the grass appeared to recover from grazings more slowly in the midsummer period than during other seasons of the year.

In addition to plant production capacity various other soil factors were determined for each soil sample taken. The oat plant material of each harvest was subjected to chemical analyses. The results of all these additional investigations will be published in further articles.

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SAWDUST COMPOSTS IN SOIL IMPROVEMENT †)

1. STUDIES ON AERATION, ACID HYDROLYSIS, MANURE, AND WASTE MATERIALS AS COMPOSTING AIDS

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As indicated in a previous paper [2]) the use of wood wastes in soil improvement has received considerable attention lately. Composting seems to offer large possibilities for avoiding the adverse effects of fresh sawdust and to utilize the potentialities of sawdust as a soil builder in the best fashion. The literature on composting of various waste products has been listed and reviewed very comprehensively in two recent publications [1]). The investigations reported here are preliminary tests of methods of composting sawdust and tests of the resulting materials on plant growth in greenhouse pot cultures.

EXPERIMENTAL

Much of the material presented here represents a development of a series of different methods requiring some detailed description upon which understanding of the results depends. Therefore they are considered together, each as a unit rather than as separate "experimental methods" and "results".

1. *Outdoor compost bins*

Four bins of rough lumber were built each to contain about 16 cubic feet. They were supported on posts above the ground and each had one louvered side and a hollow wooden cylinder placed horizontally in the center. These cylinders had numerous holes on all

†) Published with the approval of the Director of the Agricultural Experiment Station as Scientific Contribution No. 151.

*) This report is based upon portions of a thesis presented in partial fulfillment of the requirements for the degree of M.S. at the University of New Hamsphire.

four sides to further aid in aeration of the contents. Each was filled with a different mixture as follows:

Bin 1. 1/3 manure *), 2/3 sawdust.
Bin 2. 1/3 manure, 2/3 sawdust, 5 lbs urea.
Bin 3. 1/3 soil, 2/3 sawdust.
Bin 4. Rotted wood, 5 lbs urea.

The contents of these received no moisture other than that from rain and snow. The bins were filled Nov. 20, 1946.

About one year later samples were taken from each bin for analysis of plant nutrients and for some preliminary pot cultures. The analysis showed no difference in the principal plant nutrients in the contents of Bin 1 and 2 and very slight differences between Bin 3 and 4. The latter two were much lower in nutrients. Since the contents of Bin 1 and 2 were so nearly alike, plant cultures were grown only with material from Bin 1 and that from Bin 2 was omitted.

String beans and potatoes were grown in these composts. The results indicated that the sawdust had not decomposed sufficiently to support good plant growth. Probably this was due to lack of enough moisture for optimum decay, as will be emphasized later.

Compost cultures in pails. On June 1, 1949, after the composts had been standing for about 2 years more, or 3 years in all, other samples were brought into the greenhouse for nutrient tests and for further plant culture tests. The analysis results showed nitrogen, phosphorus, potassium, and pH levels all higher than before.

At this time cultures were prepared using galvanized pails of 14 quart capacity as containers for growing the plants. A hole was made in the bottom of each and the inside of the pail was previously coated with inert asphalt paint to prevent corrosion. In these cultures the contents of Bin 1 were omitted, since their properties were similar to those of Bin 2. Ten pails full of each of the three composts were prepared, along with ten pails each of fresh sawdust and of loam for comparison as indicated in Table I. The composts were used just as they came from the bins with no dilution with sand or soil. Each pail of material received 35 grams of 5-10-15 complete fertilizer mixed thoroughly in the top three inches.

*) The cow manure was already mixed with a liberal amount of sawdust used in the barn as bedding.

Four successive crops of plants were grown in these substances without renewal except for remixing the individual materials between each crop. Fertilizer was added as it appeared to be needed. The kinds of crops and the order in which they were grown were as follows: 1. oats, 100 plants per pail; 2. sunflowers, 3 plants per pail; 3. tomatoes, 2 plants per pail; 4. potatoes, one plant per pail. For yield data and criteria of the effects of the various media on plant growth, dry weights were taken of the tops of the oat plants at age 7 weeks and of the sunflowers just prior to flowering. Fresh weights were taken of tomato fruits and potato tubers. The average yields for these crops are given in Table I. In all of these cultures none of the composts supported as good growth as the soil control. An exception is found in the potato yields, where that from the sawdust-manure was best. As would be expected, the plants in the fresh sawdust gave poorest yields throughout.

TABLE I

Average Yields (grams) of Four Successive Crops in Composts					
Crops Grown	Loam	$^2/_3$ Sawdust $^1/_3$ Soil	$^2/_3$ Sawdust $^1/_3$ Manure & urea	Rotted Wood & urea	Fresh Sawdust
Oats	30.3	28.4	25.7	21.0	2.2
Sunflowers (two plants)	31.9	27.3	21.6	24.3	0.7
Tomatoes	442.3	209.0	74.7	129.7	25.3
Potatoes *)	449.5	394.1	493.1	387.5	326.1

*) All tubers weighed were over 1 inch in diameter.

Directly after harvest samples were taken of each material for nutrient analysis. The pH of all varied between 4.4 and 4.9. All nutrients were low. This series of cultures was finished May 8, 1950.

Compost cultures in crocks. On July 26, 1949, another set of compost cultures was started in one-gallon size glazed crocks, with compost fresh from the bins described above. Again they were compared with fresh sawdust and loam as control. Ten crocks were used for each material. All were fertilized equally. Two successive crops, onions and corn were grown, two plants per crock. The yields of onions were taken as fresh weight after eleven weeks and of corn as dry weight of tops after seven weeks. The corn was planted in October and did not grow well because of poor light conditions. The average yields per crock appear in Table II.

TABLE II

Average Yields (grams) of Two Successive Crops in Composts					
Crops Grown	2/3 Sawdust 1/3 Manure & urea	Loam	2/3 Sawdust 1/3 Soil	Rotted Wood & urea	Fresh Sawdust
Onions (fresh wt.) . .	147.2	124.4	93.2	88.6	3.9
Corn (dry wt.)	3.4	3.9	3.5	3.6	0.3

Dry weights of the onions were taken as well as fresh weights, but they did not alter the relative position of the yield values. The results for the onions show a slightly significant advantage for the yields in sawdust-manure as compared to controls. This is in agreement with the results on potatoes in Table I. Again there is a marked adverse effect of fresh sawdust.

Auto-irrigation cultures with compost. It seemed desirable to determine something about the response of plants in these composts where moisture is in fairly constant and abundant supply. Because of this the following experiments were performed.

Fifty porous clay pots, seven inches in diameter, were chosen and their holes stoppered. They fitted rather snugly each in a one-gallon glazed crock in such a manner that only the top flange of the pot rested on the top rim of the crock. This permitted space for water within the crock and around the outside of the porous pot nearly to the top. This space was kept full of water throughout the experiment, which permitted constant seepage of water into the soil or other media within the pot.

This type of apparatus was necessarily crude because of the lack of special auto-irrigator pots with double walls which are rather expensive [3]). Without doubt there is considerable variation in the porosity of the walls of individual ordinary flower pots. However, in order to get fifty pots of a uniform porosity many more than fifty were placed in crocks of water and allowed to stand for two days. Those that allowed close to the same amount of water to seep through were selected for the cultures.

The same kinds of materials, composts and soil, as used in the preceding experiments were placed in these auto-irrigator pots, ten pots for each material. At this time a complete fertilizer (5-10-5) was added at the rate of 9 grams per pot and thoroughly mixed with the compost or soil. Two successive crops were grown in these, sunflowers

and Swiss chard. Between the first and second crop the materials of each kind were remixed, but no additional fertilizer was added.

As the plants grew the following observations were made: growth at first was very slow, and some materials, notably the sawdust-soil compost, seemed to absorb too much moisture. The height of the sunflowers in the sawdust-manure mixture and the rotted wood exceeded that of the controls, although this relationship did not appear in the dry weights given in Table III. The plants were harvested just before flowering.

TABLE III

Average Dry Weights (grams) of Plants in Auto-Irrigator Cultures

	Loam (control)	2/3 Sawdust 1/3 Manure & urea	Rotted Wood & urea	Fresh Sawdust	2/3 Sawdust 1/3 Soil
Sunflowers	12.8	12.4	10.6	2.5	0.5
Swiss chard	2.3	13.8	9.3	0.3	1.2

During growth of the Swiss chard it was quite noticeable that the plants in sawdust-manure and in rotted wood were growing much better than the others. They were harvested after three months. Average dry weights are presented in Table III.

This ended the experimentation with this type of compost. Several conclusions may be drawn from these results. One is that this type of composting device with considerable aeration of the contents and with only moisture from natural precipitation available is not effective in producing a good growing medium for plants. Breakdown of the sawdust is very slow, and probably artificial watering would speed it considerably. There is further evidence that several successive crops grown in such compost do not decompose it to the point of being a good growing medium, as only in the last crop grown, potatoes, was there better response for one of the composts than the controls. That moisture supply in some of these composts during growth may be important is shown by the auto-irrigation cultures with Swiss chard, where two composts gave considerably better growth than the controls.

2. *Outdoor compost piles*

In another attempt to cause decomposition of fresh sawdust and make it more useful for plant growth, outdoor compost piles were

used. They were placed near a greenhouse so that additional water beyond that from rain and snow could be applied by hose. No special aerating devices were used. The use of fresh green vegetation in the form of grass silage and hen manure as active agents in composting was tested. The sawdust was largely coniferous in origin, or so-called softwood sawdust.

On July 13, 1949, five compost piles 9 by 6 feet and 3 feet high were prepared. Each was placed on tarred paper to prevent contact with the soil. Boards twelve inches wide were used to retain the sides of the piles near the bottom edges.

For mixing the ingredients of the separate piles a large metal-bottom boat or trough was used. The materials were placed in the boat and enough water was added so that the different substances came in good contact with each other. This was particularly important with the hen manure as it was obtained in a relatively pure state. A liberal amount of water and mixing was required to work it out of its lumpy consistancy and assure good contact with the sawdust. All measurements were by volume. The compost piles were of the following mixtures:

1. 1/4 hen manure, 3/4 sawdust.
2. 1/10 hen manure, 9/10 sawdust.
3. 1/2 silage *), 1/2 sawdust.
4. Pure sawdust.
5. 1/3 hen manure, 1/3 silage, 1/3 sawdust.

For the first three months temperature records of the interiors of the piles were made. The 1/2 silage- 1/2 sawdust mixture quickly reached a high level of 69.5°C. Two weeks were required for the hen manure-silage-sawdust mixture to reach a peak of 54°C. All but this latter reached the daily air temperatures by August 29, and by September 7, the No. 5 mixture came to that level. Because of the hot dry summer frequent waterings were necessary, but could not be made as liberal as desirable because of a water shortage.

Compost cultures in cans. Beginning December 20, 1949, approximately 5 months after starting the composts, samples were brought into the greenhouse for tests with plant cultures. The pH value for all these averaged about 6.6 except for the fresh saw-

*) This was silage fresh from the field as harvested and chopped in small lengths. It was mixed legumes and grass.

dust, which was 4.8. The level of plant nutrients was quite high except in the pure sawdust. Three crops were grown in these materials, radish and sunflower in separate containers, but simultaneously, and soybeans in the same media following the radish. All were grown in one-gallon size tin cans previously treated with paraffin to prevent corrosion. Drainage holes were made in the bottoms. Ten cans were used for each material, including the controls which consisted of washed sand. This was supplied a complete nutrient solution at intervals. Five radish plants and two each of the other plants were grown in each can. No fertilizer was added to the compost cultures. All were watered by hose as in conventional greenhouse practice as it appeared necessary. The yields for these cultures are given in the top portion of Table IV.

TABLE IV

Average Dry Weights (grams) of Plants Grown in Composts from Outdoor Piles

	Control	1/4 HenMan. 3/4 Sawdust	1/10 HenMan. 9/10 Sawd.	1/2 Silage 1/2 Sawdust	1/3 Hen Man. 1/3 Sil. 1/3 Sawd.	Pure Sawdust
Radish (tops)	1.48	1.21	0.68	1.35	0.94	0.30
Radish (roots)	2.53	2.64	1.25	3.15	0.86	0.41
Sunflower .	18.26	5.75	4.75	4.05	9.43	0.43
Soybean .	7.90	1.47	0.91	4.76	10.41	3.06
Auto-irrigation cultures						
Rutabaga (tops) . . .	13.87	9.70	8.57	5.38	7.58	2.85
Rutabaga (roots) . .	11.68	6.85	5.75	4.18	4.91	1.16

Auto-irrigation cultures. The same types of materials were also tested in auto-irrigation cultures set up in the same way as previously described. Here a sandy loam soil was used as control instead of sand and nutrient solution. Again no additional fertilizer was supplied. Dry weights of tops and roots at harvest appear in Table IV, bottom.

Examination of the yields in Table IV shows that in most of these cultures the control plants grew best. Exceptions are the radish roots in 1/4 hen manure-3/4 sawdust and 1/2 silage-1/2 sawdust, also the soybean yield with the hen manure-silage-sawdust mixture. It is worth noting that during growth the soybean plants in both of the hen manure-sawdust mixtures showed signs of severe nitrogen toxicity. This explains the exceptionally low yields for these cultures. As

might be expected the pure sawdust cultures were all poorest with each crop.

The principal conclusion to be drawn from this is that five months is not a long enough time to allow composting to proceed for best results with these types of mixtures. A more even supply of moisture probably would aid in rapid decomposition. Hen manure and fresh vegetation show promise of being good materials to aid in sawdust composting. The remainder of these compost piles were kept for further decay and tests at a later date.

3. *Acid hydrolysis in sawdust decomposition*

Because of the slowness of sawdust decomposition in the methods above described, others more rapid were sought. The possibilities of acid hydrolysis and heating with live steam were investigated. Fairly large volumes of sawdust, 6 to 10 gallons, were treated as follows:

1. Steamed with high pressure steam, then mixed with enough 1% H_2SO_4 to moisten.
2. Not steamed, treated with acid.
3. Treated with acid, then steamed 1 hour.
4. Treated with acid, then steamed 2 hours.

Each of these materials were stored in glazed earthenware or glass jars for 23 months at a temperature of about 65°F. The pH of the mixtures was tested from time to time and it gradually sank to below 2.0. After this period of time treatments 1 and 2 showed no signs of breakdown. Treatments 3 and 4 had become partly hydrolyzed. Five inches of liquid was present above the sawdust and a dense fungus mat grew on top of the liquid.

Excess liquid was drawn off and then each mixture was neutralized with lime suspended in water. After this, the partly acid-hydrolyzed sawdust was mixed with washed sand for growing plants, in the proportion of 3 parts sawdust to one of sand. Enough of each was prepared to fill 10 one-gallon glazed crocks. For controls 10 crocks were filled with sand only. Each set of cultures received a complete nutrient solution from time to time. Two successive crops were grown in these materials, Swiss chard and rutabaga, two plants per crock. Tops of the chard were harvested for dry weight determinations after 3 months and tops and roots separately for the rutabaga after 2 months. The control plants grew far more vigorously than the others with both

crops as is evidenced by the average dry weights given in Table V.

TABLE V

Average Dry Weights (grams) of Plants Grown in Acid-Hydrolyzed Sawdust

	Control	Treatments			
		1	2	3	4
Swiss Chard	6.28	0.16	0.21	0.49	1.05
Rutabaga (tops)	7.88	2.80	2.58	4.44	4.91
Rutabaga (roots) . . .	3.66	0.96	1.13	1.39	1.88

The conclusions that may be drawn from these results are: 1. The various acid-hydrolysis and steaming treatments did not break down the sawdust to any great extent; 2. The resulting materials were quite adverse to plant growth with the first crop; 3. In the second crop the adverse effect was somewhat less.

4. *Indoor composts in jars — fish waste and green vegetation*

The purpose of this experiment was to test other organic materials as aids in sawdust decomposition under more uniform conditions indoors. The opportunity arose to secure fish waste in the form of partly decayed sardines. Fifty pounds of these were obtained in the frozen condition. They were ground in a food chopper and then mixed with sawdust in the following proportions, by volume;

1. 5% fish, 95% sawdust.
2. 10% fish, 90% sawdust.
3. 20% fish, 80% sawdust.
4. 25% fish, 75% sawdust.

Six gallons of each of these mixtures were prepared and stored in large glass battery jars in the greenhouse. The contents were well moistened, then covered to prevent evaporation. Each mixture was stored for about 3 months. The odor from them became very strong as time went on.

At close to the same time of preparation of the fish mixtures, a mixture of one half finely ground fresh tomato foliage and one half sawdust was prepared and stored in a 10 gallon-size glazed crock. A similar crock was filled with fresh sawdust and both were kept moist and stirred occasionally. These also were stored for 3 months in the greenhouse.

The pH of these mixtures at the end of the storage period varied

between 8.22 and 8.75 for the fish-sawdust, increasing with the percentage of fish. The fresh sawdust was unaltered in appearance and had a pH of 4.52. The vegetation-sawdust pH was 7.70. It had a fungus mat on top and was very dark in color. In the fish mixtures there was considerable decomposition as evidenced by a shrinkage in volume proportional to the amount of fish. Cultures of soybeans were grown in these materials in gallon-size cans. Because of shrinkage in volume only 8 cans of each fish mixture could be filled. They were used undiluted with soil or sand. The other materials each filled 10 cans. Growth was rather poor in all of the fish mixtures as seen from the dry weight data in Table VI.

TABLE VI

Average Dry Weights (grams) of Soybeans in Fish-Sawdust Compost and Green Vegetation-Sawdust Compost

Loam (Controll)	Green Veg. 50/50 Sawdust	Fresh Sawdust	Parts fish to parts sawdust			
			5/95	10/90	20/80	25/75
3.99	1.01	0.87	0.75	0.73	0.69	0.83

At first toxicity symptoms appeared in the plants growing in the fish mixtures, probably due to ammonia and partly to the high pH. Later this disappeared and a nitrogen deficiency was seen. Germination was slow in these mixtures. As all of the plants in the fish mixtures yielded more poorly than even those of the fresh sawdust, it seems that this kind of compost is not a good one to use. The green vegetation-sawdust mixture might be worth further trial with longer periods allowed for composting and perhaps with fertilizer combinations.

5. *Sawdust-soil cultures. Sodium nitrate versus urea*

In order to gain some information about the relative merits of sodium nitrate and urea as sources of nitrogen for plants growing in sawdust-soil mixtures, cultures were prepared, each mixture in 10 pails, as follows:

1. Urea, 10 grams per pail, 1/4 sawdust-3/4 soil.
2. Urea, 10 grams per pail, soil.
3. $NaNO_3$, 10 grams per pail, 1/4 sawdust-3/4 soil.
4. $NaNO_3$, 10 grams per pail, soil.
5. Soil only.

All except group 5 received adequate amounts of additional nutrients. Oat seed, 100 per pail was planted. For three weeks the plants grew well and uniformly in all treatments. Then differences appeared. The control plants, soil only, appeared most vigorous, but the soil dried most quickly of all. The nitrate treated plants were next, while those with urea were stunted and blue green in color. After 9 weeks the plants were harvested for dry weights as given in Table VII.

TABLE VII

Average Dry Weights (grams) per 100 Plants of Oats Grown in Sawdust-soil mixtures. Urea versus Nitrate

$NaNO_3$ $^1/_4$ Sawdust $^3/_4$ Soil	$NaNO_3$ Soil	Urea $^1/_4$ Sawdust $^3/_4$ Soil	Soil Only	Urea Soil
19.18	18.17	15.97	12.82	5.74

It appears from these results that a nitrate is a better source of nitrogen for plant growth than urea either with or without sawdust. Urea in smaller concentrations might have further possibilities in sawdust-soil mixtures.

Acknowledgement

Thanks are here expressed to the personnel of the Engineering Experiment Station of the University of New Hampshire, who under the direction of Dean L. E. Seeley have aided these investigations in various ways.

SUMMARY

1. Different sawdust mixtures with cow manure and soil were composted in bins with aeration for three years. Rotted wood was also tested. No moisture was added other than natural precipitation. Greenhouse pot cultures of four successive crops and two other crops showed these composts not decomposed sufficiently to support good growth. Auto irrigation cultures further confirmed this although here one crop grew better in two of the mixtures than controls. From this it appears that moisture is a very important factor in decomposing sawdust and that normal precipitation may not be enough.

2. Compost piles of sawdust were built outdoors near a water supply. Various mixtures of sawdust with hen manure and fresh grass silage were prepared. These were allowed to stand for 5 months. At the end of this time

greenhouse pot culture trials with various plants revealed that some mixtures have promise of being good growth media. These were notably some of the hen manure, green vegetation, and sawdust combinations. It was evident that a longer time of composting is necessary for best results. Further tests are being made with these mixtures.

3. The use of acid-hydrolysis and steam as a means of sawdust decomposition, with subsequent pot culture tests with such material revealed that this is not an effective method for producing good growing media for plants.

4. Fish waste mixtures with sawdust composted in jars indoors gave rapid breakdown of the sawdust. The resulting materials undiluted with soil did not produce good plant growth. The same was true of finely chopped green vegetation with sawdust composted under the same conditions. Breakdown here was much slower than in the fish mixtures.

5. Comparisons were made of urea to nitrate as a source of nitrogen in growing plants in soil with and without sawdust. Plants grown in pot cultures of such mixtures gave higher yields with nitrate.

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Plant and Soil IV, no 3 January 1953

SAWDUST COMPOSTS IN SOIL IMPROVEMENT †)

II. POT CULTURE STUDIES WITH COMPOST MIXTURES OF SAWDUST AND MANURE, STEAM TREATED COMPOSTS, AND MISCELLANEOUS MIXTURES

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The experimental work here reported is a continuation of the preliminary tests of methods of sawdust composting reported previously [3]). Results are given of further pot culture tests with plants in composts mentioned there after longer periods of decomposition. Information also was sought on effects of treatment with high-pressure steam on sawdust-manure mixtures and on effects of varying proportions of sawdust to soil on plant growth.

EXPERIMENTAL

1. *Further tests on material from outdoor compost piles.*

Since the compost described in part 2 of the previous paper in this series [4]) had shown some promise of producing good material in which to grow plants, further cultures were grown in them. The mixtures used in these composts were as follows:

1. 1/4 hen manure, 3/4 sawdust.
2. 1/10 hen manure, 9/10 sawdust.
3. 1/2 silage (fresh grass and legumes), 1/2 sawdust.
4. Pure sawdust.
5. 1/3 hen manure, 1/3 silage, 1/3 sawdust.

The preliminary tests of these materials in plant cultures mentioned

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*) This report is based upon portions of a thesis presented in partial fulfillment of the requirements for the degree of M.S. at the University of New Hampshire.

above had been done after composting for 5 months. Apparently, this was not enough time for optimum results. They were allowed to stand for 7 months more. Early in July 1950, about a year after the the piles were built, portions of each were taken for other cultures. For containers wooden boxes of about 1 bushel or 35 liters capacity were used. This allowed considerably more space for root growth than the pails or crocks hitherto used. Since greenhouse conditions at this time of year were too hot for best growth of plants, the first crop, corn (maize), was grown outdoors. The boxes were placed on gravel, for good drainage, near the greenhouse so that water could be readily supplied.

Ten boxes full of each compost listed above were prepared. Besides this there were 10 boxes of soil as a control. Each box received 10 grams of 5-10-10 fertilizer mixed uniformly throughout the contents. The positions of the boxes of any one material was replicated or distributed throughout the area so as to avoid positional effects as much as possible. Eight seeds were planted per box, later thinned to five seedlings. The composts were used just as they came from the piles, without dilution with soil or sand.

From the start the plants in the one third each hen manure-silage-sawdust mixture grew best. In fact, they far outgrew the next best which were those in the soil controls. All the others were much poorer in growth, some being of light color and stunted.

After growing for 50 days the tops of the corn were harvested and dry weights taken. The averages of these based on 5 plants per box are shown in Fig. 1. There outstanding size and vigor of the plants in the manure-silage-sawdust mixture is emphatically substantiated. The yields for the other composts are all significantly less than those of the controls. After harvest samples of the composts and soil were taken for pH and nutrient analysis, which showed pH variation between 5.7 and 6.9. The nitrogen was low in all cultures, but the phosphorus and potassium medium in amounts. Magnesium was high in all cultures.

Three successive crops were grown in these same materials following the corn, two of soybeans and one of potatoes. These were grown in the greenhouse as the first crop of soybeans was not planted until late in September. Before planting each crop an adequate equal amount of complete fertilizer was added to each container, except before the last crop, potatoes. In this instance the amounts of certain

nutrients varied so much from one mixture to the next, that an effort was made to supply only those elements markedly deficient. Another modification was made in growing the potato crop with respect to containers. The volumes of certain mixtures had decreased considerably from decay, so that for the potatoes all the materials were transferred to 14 quart-size pails. Enough was available for 10 pails of each material.

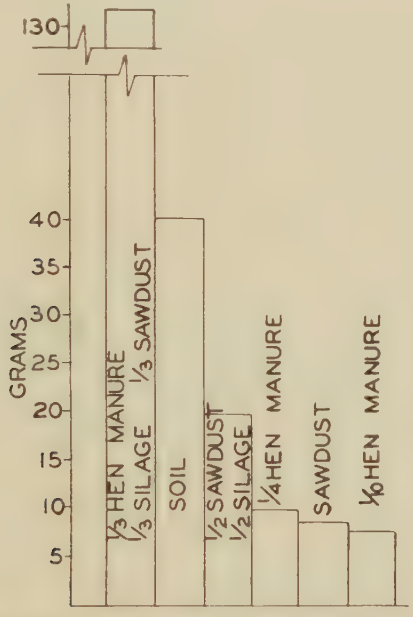

Fig. 1. Average dry weights of 5 corn plants per wooden box of compost.

In preparation for the third crop it was noticed that the mixtures in the 1/4 hen manure-3/4 sawdust and the manure-silage-sawdust were completely broken down as regards recognition of any of the original ingredients. This was the first positive evidence that we have had that the structure of fresh sawdust may be completely disintegrated in a short period of time. This process probably was hastened to a large extent in its later stages by the regular supply of water and higher temperatures in the greenhouse because the same mixtures in the piles outside still retained much of their original structure. A very large factor also was nitrogen supply, as evidenced by the very slight breakdown in the pure sawdust and in the 1/10 hen manure-sawdust mixture.

The average dry weights of tops and roots for 5 plants (number grown per box) for the first crop of soybeans and of the tops for the second crop are given in Table I.

TABLE I

Average Dry Weights (grams) of Soybean Plants (5 plants per unit) Grown in Composts from Outdoor Piles

Crop	Soil	1/2 Silage 1/2 Sawdust	1/3 Sawd. 1/3 H.Man. 1/3 Silage	1/4 H.Man. 3/4 Sawd.	Fresh Sawd.	1/10 H.Man. 9/10 Sawd.
Soybean 1st crop						
Tops	9.4	6.9	3.6	2.5	1.7	1.4
Roots	10.0	29.0	12.0	7.5	6.2	4.0
Soybean 2nd crop						
Tops	7.2	8.5	7.6	8.1	2.7	3.4

In the yields of tops for the first crop of soybeans the plants in the soil produced considerably more than any of the composts. However, two of the compost grown cultures show better root development than the controls. In the second crop the yields of tops for three of the composts are slightly above that of the controls.

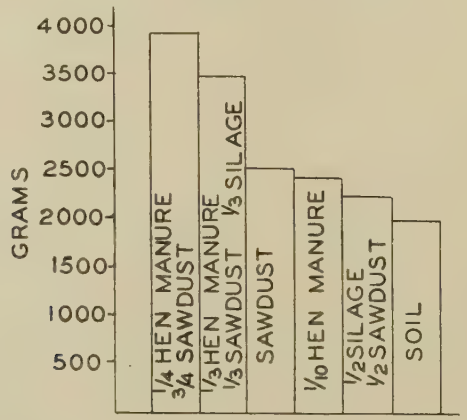

Fig. 2. Total yields of potato tubers grown in composted materials.

The yields in total fresh weights of tubers for the potato crop are given in Fig. 2. They were harvested after 86 days of growth. It is interesting to note that all of the composts, even the pure sawdust, produced greater yields than the soil controls. Only two of them, the

manure-silage-sawdust mixture and the 1/4 manure-3/4 sawdust yields, are significantly greater than that of the controls. In this respect the production of the manure-silage-sawdust is notably consistant with the high yields of corn produced by it in Fig. 1. It would seem that this type of mixture shows a great deal of promise and is worth further investigation.

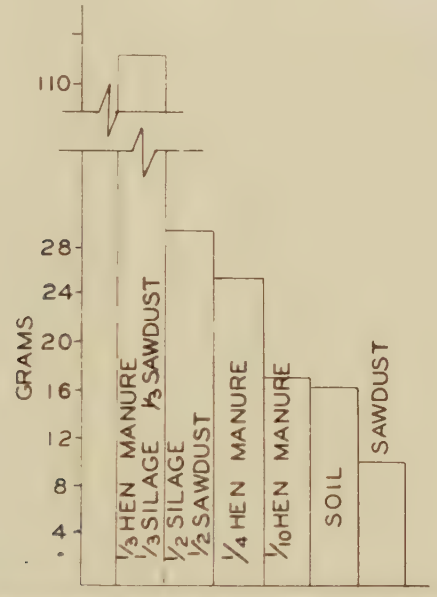

Fig. 3. Total dry weights of tops of cabbage plants grown in composts.

A further test of these materials was made later in the winter, to compare the degree of breakdown of the sawdust mixtures directly from the outdoor piles with that of those above described. On Feb. 16, 1951 enough of each was obtained to fill 10 one-gallon size cans. Soil was again used as control. Five grams of 5-10-10 complete fertilizer was thoroughly mixed with the contents of each can. Young cabbage plants were transplanted to these cultures, one plant to each can. After slightly over two months growth the tops of the plants were harvested for dry weights, which are presented in Fig. 3. Again the yield for the soil cultures was poorest of all, and that of the manure-silage-sawdust outstandingly the best. Two others, the 1/2 silage-1/4 sawdust, and the 1/4 manure-3/4 sawdust gave yields significantly greater than the controls.

Any explanation of why the manure-silage-sawdust mixture should be so far superior to the other cultures would be debatable. The analysis showed that this mixture had the highest pH after the cabbage crop and the most available nutrients present, except for the soil. None of the other composts had any available nitrogen left. It is probable that this compost had decomposed more completely than the others and that the resulting increase in material of a colloidal nature helped to retain the available nutrients. This questions is of considerable interest in connection with the investigations reported by Ingham [2]). The author presents evidence which shows that cellulosic and related materials, such as are found in plant residues and humus, can adsorb nitrogen from the air. This and other compounds carried into the soil by successive rains may be important adjuncts to the maintenance of soil fertility.

Auto-irrigation cultures. These same materials were tested for plant growth in two sets of auto-irrigation cultures, parallel in time with the cultures described above. The procedure for one set was as follows: 42 one-gallon glazed crocks with a one inch hole in the bottom of each were set on top of 42 other one-gallon crocks filled with water. A porous carbon tube one foot long was inserted half-way into each water crock, and the other half into the cultures which were in the top crock. Rubber tape was used to fill the hole tightly between the crocks to prevent the roots from the cultures from penetrating into the water in the lower crocks. This was an attempt to keep the media uniformly and constantly supplied with water, as used by the plant, by means of the carbon tube. Each tube was stoppered tightly at both ends after being completely filled with water. The water level in the tubes remained close to the top throughout the experiment. No other water was supplied to the cultures throughout growth, except that the soil or composts were thoroughly moistened at the start. Composts from the same sources as mentioned above in comparison to soil were used and seven culture jars set up for each variable. Ten grams of 5-10-10 fertilizer was mixed with the contents of each culture jar. The plants grown were Mammoth Russian sunflower. They were started at five seedlings per crock, later thinned to two. The plants grew very well in all the treatments and after 48 days were harvested for dry weight determinations of the tops. These are presented in Table II.

TABLE II

Average Dry Weights (grams) of Two Crops Grown in Composts with Auto-irrigation						
Crop	1/2 Silage 1/2 Sawd.	1/3 H. Man. 1/3 Silage 1/3 Sawd.	1/10 H.Man. 9/10 Sawd.	Soil	1/4 H. Man. 3/4 Sawd.	Fresh Sawd.
Sunflowers . . .	24.5	24.0	19.5	15.0	14.4	8.0
Corn	16.5	13.5	12.9	10.2	11.5	8.9

Another set of cultures was grown with the type of auto-irrigators described previously [4]), in which porous flower pots were placed inside crocks of slightly larger diameter. The same types of materials as in the set above described were used to fill these pots to the extent of seven per treatment. The contents of each pot were thoroughly mixed with 3.5 gram of 5-10-10 fertilizer. Field corn was grown in these, two plants per pot. All grew more vigorously than the sunflowers; partly due to better light conditions prevalent in the spring. The plants were harvested for dry weights of the tops after 52 days of growth. Results appear in Table II.

These results were in general agreement with those of the cultures described above in showing best yields for certain of the composts with either fresh vegetation or hen manure or both as a composting agent mixed with sawdust.

Certain observations concerning the limitations of these auto-irrigation methods made during the course of these experiments may be worth recording. The chief characteristic encountered was the high water content of the compost mixtures. This seemed to be more pronounced in the finer textured composts. It may be an advantage under certain environmental conditions, under others a disadvantage. At certain times of the year, when light intensity is great and the atmospheric stress or "pull" of evaporation is great, the auto-irrigation cultures may grow very well. At times, the supply of moisture may be low enough to cause wilting under such conditions. The pH of the sawdust mixtures showed an increase as was found by McCool [3]) when in his observations he noted a rise in the pH when sawdust was leached with distilled water.

2. *Indoor composts of steam treated sawdust-manure mixtures.*

Several sawdust-manure mixtures were composted in the greenhouse in large crocks after being subjected to high pressure steam in an autoclave. The intention was not to test the effect of sterilization,

for obviously the mixtures would become contaminated as soon as cooled. Rather, it seemed possible that the sawdust might be altered in such a way as to absorb more of the liquid portion of the manure and to break down more easily. For three of the mixtures the sawdust only was placed in the autoclave and treated with steam at 20 pounds pressure and 120°C for one hour. The hen manure was mixed with liberal amounts of water until the resulting mixture was a thick liquid. Immediately after removal from the autoclave the hot sawdust was mixed with the manure thus prepared in three different proportions as follows:

1/3 hen manure, 2/3 sawdust.
2/10 hen manure, 8/10 sawdust.
1/10 hen manure, 9/10 sawdust.

These proportions were based on the volume of manure before dilution with water. Another mixture of 2/10 hen manure-8/10 sawdust was prepared and both steamed together after mixing.

These were allowed to stand for 50 days and then were tested in plant cultures, in comparison to soil only as control. Ten tin cans of one-gallon size were filled with each compost which was first mixed with soil at the rate of 1 part compost to 3 of soil. Ten cans of soil only for controls were prepared by mixing thoroughly of 5 grams 5-10-10 fertilizer in the contents of each can. No fertilizer was added to the compost mixtures.

Radish plants were grown in these cultures, 4 plants per can. Foliage growth was quite luxuriant, probably due to abundant nitrogen. After about one month of growth quite large roots were evident on some plants and they were harvested. At this time pH and nutrient analysis was made of samples of the soil and composts. The pH for the soil was 4.7 and alle the others were close to 5.4. Nitrogen was low to medium in the soil, high in the 1/3 manure mixture and low in the others. Phosphorus was low to medium in all, and potassium high in all. The average dry weights for the tops and roots are given in Table III.

All of the plants grown in compost mixtures produced higher yields than those in soil. The 1/3 manure-2/3 sawdust mixture showed best results in both tops and root yields, probably because of the high level of nutrients in the manure. Any of these types of treatments seems worth further investigation.

TABLE III

	Average Dry Weights (grams) of Radish Plants Grown in Composts of Steamed Sawdust-Manure Mixtures				
	1/3 Man. 2/3 Sawd.	2/10 Man. 8/10 Sawd. (Steamed together	1/10 Man. 9/10 Sawd.	2/10 Man. 8/10 Sawd.	Soil
Roots.	0.47	0.46	0.45	0.40	0.25
Tops.	3.44	2.28	3.05	2.61	1.80

3. *Pot cultures with sawdust-soil mixtures in varying proportions.*

In order to gain some information on the optimum quantity of either fresh or rotted sawdust which may be added to soil for best plant growth these cultures were started. The rotted sawdust was from the same source, a large 10 year old pile, as was used in the field plots previously reported [1]). Enough of each mixture was prepared to fill eight gallon-size tin cans. All proportions were on a volume basis. No fertilizer was added to any of these cultures before the growth of the first crop. The various cultures were prepared as follows:

Culture No.

1. 100% fresh sawdust.
2. 80% fresh sawdust-20% soil.
3. 60% fresh sawdust-40% soil.
4. 40% fresh sawdust-60% soil.
5. 20% fresh sawdust-80% soil.
6. 10% fresh sawdust-90% soil.
7. 100% soil.
8. 100% rotted sawdust.
9. 80% rotted sawdust-20% soil.
10. 60% rotted sawdust-40% soil.
11. 40% rotted sawdust-60% soil.
12. 20% rotted sawdust-80% soil.
13. 10% rotted sawdust-90% soil.

Two successive crops were grown in each of these mixtures, Swiss chard-1 plant per can, and radish-4 plants per can. Between the two crops an application of fertilizer was made of 5 grams of 5-10-10 fertilizer per can. The Swiss chard was harvested after 7 weeks and the radish after one month of growth. The average dry weights appear in Table IV.

TABLE IV

Average Dry Weights (grams) of Two Crops Grown in Various Sawdust-Soil Mixtures

Culture Number												
1	2	3	4	5	6	7	8	9	10	11	12	13
Swiss Chard												
2.6	2.8	2.5	0.8	0.9	1.8	3.3	1.6	2.3	3.4	2.7	2.8	2.9
Radish Roots												
4.2	4.7	5.3	5.5	5.7	4.9	4.8	4.2	4.7	5.1	4.9	4.5	5.3
Radish Tops												
4.5	4.3	5.0	5.1	5.4	5.1	5.3	4.5	4.8	5.2	5.5	4.9	5.7

The Swiss chard showed a definitely poorer growth in fresh sawdust mixtures compared to the rotted sawdust. Practically all workers have found that fresh sawdust when first mixed with soil has a depressive effect on plant growth due to a nitrogen deficiency created by the activities of the micro-organisms causing decay of the sawdust. In the second crop the radish plants showed a definite improvement in yields in the fresh sawdust treatments, which was due partly to decay of the sawdust during the growth of the first crop and partly to the nitrogen added in the fertilizer. It is not possible to draw from these results any definite conclusions regarding the optimum amounts of sawdust to add to soil for plant growth. This is because of several exceptions and irregularities in the results and because many of the yields were so close together as to lack significance. In general it appears that mixtures of varying proportions between 10 and 40% sawdust with corresponding proportions of soil may be best. Other factors, such as variations in moisture supply in culture such as these, may overshadow the effects of the sawdust-soil proportions. This point needs further study.

4. *Pot cultures with pure sawdust compared to pure soil.*

This set of cultures was started with the purpose of testing plant growth in pure sawdust compared to soil when abundant nitrogen and other nutrients were supplied. The large 14-quart pails in which some other cultures were previously grown were used for containers. Ten pails were used for each of the following materials: fresh sawdust rotted sawdust, soil. The contents of each pail was mixed with 20 grams of 5-10-10 fertilizer. One tomato plant was grown in each pail. As growth progressed additional amounts of fertilizer were

applied as deficiencies appeared. Blossoming of the plants was earlier on those in sawdust than those in soil, but blossom end-rot was more prevalent on the fruits of the sawdust-grown plants. Fresh weights of the ripe fruit were recorded as they ripened for yield data. The average yields of fresh fruits were:

Soil	*Fresh Sawdust*	*Rotted Sawdust*
325 g	240 g	200 g

These results indicate that pure sawdust, whether fresh or rotted, is not a good medium in which to grow tomatoes, even with plenty of nutrients.

5. *Pot cultures with bark and shavings.*

In the autumn of 1950 some various types of waste bark became available for experimentation. These were by-products from a paper-pulp industry in northern New Hampshire. During the summer of 1950 several compost piles were started with various mixtures of birch shavings. Further work is planned with these, but after 3 months time samples of two of these materials were brought into the greenhouse for tests in preliminary cultures. Ten each of the gallon-size tin cans were filled with the following materials (70 cans in all):

1. fresh sawdust.
2. birch shavings.
3. soil.
4. 1/2 green vegetation, 1/2 shavings.
5. old softwood bark.
6. old hardwood bark.
7. 1/2 fresh sawdust, 1/2 old softwood bark.

A large part of the different kinds or bark was quite finely divided. Ten grams of 5-10-10 fertilizer was thoroughly mixed with the contents of each can before planting. Young cabbage plants were transplanted to these, one to each can. Additional electric light for 5 hours daily was supplied the plants during most of December and January. During growth the plants in the birch shavings had the poorest appearance, followed by those in fresh sawdust and in the shavings-vegetation mixture. After almost 3 months growth the tops of the plants were harvested for dry weights as given in Table V.

TABLE V

Average Dry Weights (grams) of Cabbage Plants Grown in Waste Bark and in Shavings Composts						
1	2	3	4	5	6	7
2.64	1.69	5.11	3.2	8.78	7.09	6.74

From this it is evident that neither of the shavings composts nor the sawdust produced as good growth as the soil. All of the different kinds of bark produced yields greater than that of the soil, the old softwood bark significantly so. It would seem that these materials are worth further investigation.

Acknowledgement. Again thanks must be expressed to the Engineering Experiment Station of the University of New Hampshire for aid in this work.

SUMMARY

1. Results reported previously had indicated that five months of composting was not sufficient for various mixtures of sawdust with hen manure, with green vegetation (silage), or both, to produce good plant growth. Further plant cultures with these after one year had elapsed produced significantly greater yields with some composts than with soil controls for several crops. Outstanding in this respect was the mixture of sawdust-manure-silage in equal parts, although the mixture of 3 parts sawdust to 1 of manure also produced some good yields.

Especially noteworthy was the fact that this length of time for composting plus the effects of the growth of two or three successive crops in the greenhouse produced a material almost like soil in appearance, with no recognizable portions of the original materials remaining.

Auto-irrigation cultures with these materials in general confirmed these results. It was observed also that certain of the composts would absorb water from the auto-irrigator pots to a much greater extent than did soil.

2. Sawdust-hen manure mixtures were prepared in which the sawdust was first treated with high-pressure steam. After standing for almost two months all of these mixtures produced better growth of one crop in pot cultures than the soil control.

3. Pot cultures of plants grown in sawdust-soil mixtures (both rotted and fresh sawdust) of varying proportions gave yield data which were inconclusive as to optimum combinations of these. Apparently other factors such as variations in water supply (if this is not known or accurately controlled) may affect plant growth to a greater extent.

4. Pure sawdust, whether rotted or fresh, did not produce as good tomato yields as soil, even when abundant plant nutrients were present.

Here again, water relations may be a primary factor, as the retention of water by either of these materials in the pure state was not as great as that of soil.

5. Preliminary cultures of plants with various partly composted shavings mixtures and with waste bark gave good growth in the latter and indicated some potentialities for it. The shavings mixtures were not composted long enough for best results.

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COUNTING OF AZOTOBACTER IN SOILS

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The three methods generally used for counting Azotobacter in soils are:

1) Plating on mannitol agar,
2) the dilution method and
3) plating on silica gel.

Though mannitol agar is a selective medium, being free of N, many other organisms may still grow on this medium and despite the characteristic appearance of the Azotobacter colonies (large, limpid and opaque) it is often idifficult to distinguish them on thick plates. Further difficulties in the identification of embedded colonies make this particular method not only a difficult one, but also rather inaccurate.

The dilution method is very laborious and requires a good deal of apparatus — hence it is not applicable on a large scale. Similar criticisms apply to Winogradsky's [2]) silica gel method.

It was found by Winogradsky [2]) that benzoic acid and benzoates are readily utilized by Azotobacter as a source of energy and using sodium benzoate at a concentration of about 0.3%, he was able to isolate Azotobacter from soil. Because of the antiseptic action of sodium benzoate, other soil organisms are greatly suppressed and Azotobacter may be isolated in a fairly pure state. The colonies on this benzoate medium are highly characteristic and black in colour. Starch and dextrin are also utilized as energy sources and in culture medium, also impart a black colour to the colonies.

In view of the fact that on benzoate, starch and dextrin media, Azotobacter colonies are black in colour, it was decided to try such

media in the counting of Azotobacter. The medium was prepared from a solution of the following composition:

KH_2PO_4	0.2 g
$MgSO_4$	0.2 g
NaCl	0.2 g
$CaSO_4$	0.1 g
$FeSo_4$	0.01 g
Na_2MoO_4	0.05 g
$MnSo_4$	0.05 g
Tap water to 1.000 cc	

Sodium benzoate, starch and dextrin were added at 0.2, 1.0 and 1.0% concentrations respectively. Soil was plated on all three media and counts made after six days' incubation at 30°C. The results given in Table I show that sodium benzoate gives the highest count of Azotobacter colonies compared with other media where overwhelming numbers of other micro-organisms were also present. It is possible that in these media, weaker cells are suppressed thus leading to a low count.

TABLE I

Showing the Azotobacter counts on various media.

Set No.	Medium used	Dilution	Plate counts	Average
1. *a.*	0.2% Sodium benzoate	1/10	215, 235, 240	230
b.	1.0% Starch	1/10	105, 151, 120	125
c.	1.0% Dextrin	1/10	108, 120, 112	113
2. *a.*	0.2% Sodium benzoate	1/10	180, 212, 200	197
b.	1.0% Starch	1/10	85, 94, 76	85
c.	1.0% Dextrin	1/10	100, 86, 95	94
3. *a.*	0.2% Sodium benzoate	1/10	225, 240, 250	238
b.	1.0% Starch	1/10	108, 112, 95	105
c.	1.0% Dextrin	1/10	115, 120, 111	115

It was also observed that 1) the black colour appeared much earlier in the benzoate medium than in the other media, 2) the intensity of the black colour was greatest in the benzoate medium. Although sodium benzoate is readily utilized by Azotobacter as a source of energy, it is known to have an antiseptic action on micro-organisms and therefore the effect of different concentrations of this salt was investigated. Accordingly counts were made on a number of media containing different concentrations of sodium benzoate and the results (Table II) show that:

1) 0.1 and 0.2% sodium benzoate gave equal counts,

2) 0.5 and 1.0% sodium benzoate are decidedly toxic to Azotobacter. In two sets the plates were found blank, but in one case some colonies had developed though the count was low.

TABLE II

Showing the effect of different concentrations of sodium benzoate on the Azotobacter count.

Set No.	Medium used	Dilution	Plate counts	Average
1. *a.*	0.1% Sodium benzoate	1/10	250, 301, 275	275
b.	0.2% ,, ,,	1/10	245, 235, 241	240
c.	0.5% ,, ,,	1/10	30, 35, 22	29
d.	1.0% ,, ,,	1/10	20, 15, 17	17
2. *a.*	0.1% Sodium benzoate	1/10	250, 225, 235	235
b.	0.2% ,, ,,	1/10	245, 297, 275	272
c.	0.5% ,, ,,	1/10	Blank	—
d.	1.0% ,, ,,	1/10	Blank	—
3. *a.*	0.1% Sodium benzoate	1/10	237, 295, 285	273
b.	0.2% ,, ,,	1/10	235, 290, 290	272
c.	0.5% ,, ,,	1/10	Blank	—
d.	1.0% ,, ,,	1/10	Blank	—

To verify these results, further counts were made on 0.1 and 0.2% benzoate media. The results (Table III) show that:

1) media containing 0.1 and 0.2% sodium benzoate in most cases gave nearly equal counts though in one case, the 0.1% medium gave a rather higher count,

2) the colour of the colonies was slightly darker in the 0.2% media.

TABLE III

Showing the counts on 0.1 and 0.2% benzoate media.

Set No.	Medium used	Dilution	Plate counts	Average
1. *a.*	0.1% benzoate	1/10	225, 202, 215	214
b.	0.2% benzoate	1/10	212, 206, 185	201
2. *a.*	0.1% benzoate	1/10	178, 247, 213	213
b.	0.2% benzoate	1/10	167, 140, 166	158
3. *a.*	0.1% benzoate	1/10	225, 195, 207	209
b.	0.2% benzoate	1/10	219, 245, 184	216

On the basis of the above experiments it is recommended that 0.1% sodium benzoate be used in the counting of Azotobacter in soils. This medium has the following advantages:

1) contaminants are greatly suppressed,

2) the colonies of Azotobacter are bigger in size than those of the contaminants and being black in colour are easily identified.

SUMMARY

A new medium has been evolved for counting Azotobacter by the plating method. The advantages of this medium are:

1) contaminants are greatly suppressed,

2) the colonies of Azotobacter are bigger in size than those of the contaminants and being black in colour are easily identified.

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ON PHYTIN PHOSPHORUS IN THE SOIL

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1. INTRODUCTION

Many investigations by a number of workers have shown that a great part — often about half — of the phosphorus in the soil is found in organic compounds.

As the organic compounds of phosphorus in the soil are decomposed with great difficulty, it is generally assumed that their importance for the supply of phosphorus to the plants is slight. For this reason the inorganic compounds of phosphorus of the soil have been objects of much greater interest than the organic compounds. In nearly all methods for the determination of phosphorus deficiency they are not included.

As the organic phosphorus compounds of the soil are decomposed with great difficulty, it was generally assumed till about 1940 that they mainly consisted of nucleic acids or similar compounds. Indeed several workers have succeeded in showing the presence of such components of the nucleic acids as for instance bases of purine or pyrimidine, pentoses, and phosphoric acid in impure precipitates isolated from soil extracts, whereas nobody has ever succeeded in isolating pure nucleic acids from the soil.

In attempts to isolate organic phosphorus compounds from the soil the procedure has nearly always been as follows: the soil is first leached with dilute acid in order to remove Ca^{++}, which forms salts which are insoluble in basic fluid with most organic phosphorus compounds. The acid-leached soil is treated with ammonia water or

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a sodium hydroxide solution, by which means the greater part of the organic substance in the soil, together with some inorganic substances is decomposed. After filtration attempts are made by various methods to remove from the basic solution inorganic substances, humic acids, etc.

Wrenshall and McKibbin[17]), who used ammonia water as an extracting agent, tried to isolate nucleic acid in the following way: the surplus of ammonia was removed from the ammonia solution under vacuum, after which the solution was made slightly acid by means of hydrochloric acid. In this way a precipitate ("humic acid") was formed, which was filtered off and washed with hydrochloric acid. The precipitate was dissolved in ammonia water and was precipitated with hydrochloric acid. This process was repeated once more, after which the filtrate and washings were concentrated under vacuum, acidified with concentrated hydrochloric acid and precipitated with 4–5 volumes of alcohol. The precipitate so formed was filtered off and washed with alcohol. This "nucleotide preparation" gave on hydrolysis pentose, phosphoric acid, and the bases adenine and uracil.

Wrenshall, Dyer and Smith[15]), however, found that the "precipitate of nucleic acid" had a lower content of nitrogen and was more resistant to decomposition in the soil than nucleic acids and thus could not consist of pure nucleic acids. As they assumed that this might be due to an admixture of phytin, they oxidized the precipitate of nucleic acid with bromine in a basic solution (phytin is resistant to this oxidation), and after filtering off a precipitate which had been formed, they made the solution acid with hydrochloric acid and precipitated it with ferric chloride. The result was a yellowish brown precipitate which looked like ferric phytate and had nearly the same ratio between Fe and P. A qualitative test showed inositol to be present.

Wrenshall and Dyer[14]) purified a precipitate which had been obtained in nearly the same way, as follows: the precipitate was decomposed with sodium hydroxide, the ferric hydroxide formed was filtered off, and the filtrate was oxidized with bromine and then extracted with ether, a colourless solution thus being obtained, which after being acidified with hydrochloric acid and precipitated with ferric chloride gave a precipitate which completely resembled ferric phytate. The ratio Fe/P in the precipitate was 0.76, while in

ferric phytate obtained by precipitation of phytin from wheat bran under the same conditions it was 0.71.

Yoshida[18]) in 1940 isolated a sodium salt which contained phosphorus and which by hydrolysis yielded 100 mg pure inositol.

In 1945 Bower[1]) stated that he had identified phytin in a sodium hydroxide extraction from soil. He isolated a ferric salt the P and inositol contents of which were determined quantitatively. The ratio inositol/P in isolations from three soils was found to be 0.974, 1.009 and 0.976 respectively. In phytin this ratio is 0.968. Furthermore Bower showed the presence of lower inositol phosphoric acid esters in the solution.

2. CHEMISTRY OF PHYTIN

The following account is mainly based on a survey by P. Fleury and P. Balatre, *Les inositols*, Paris 1947.

Phytin, $C_6H_6(OPO_3H_2)_6$, is the hexaphosphoric acid ester of the hexavalent alicyclic alcohol mesoinositol.

It is a strong 12-basic acid, which in many of its properties resembles phosphoric acid, but is considerably stronger[5]). It is a thick and sticky yellowish fluid soluble in water, insoluble in ether, benzene, and chloroform.

The salts resemble the salts of phosphoric acid, but as a rule are more difficult to dissolve.

Sodium phytate, $C_6H_6(OPO_3)Na_{12}$, is very easily soluble in water, but insoluble in alcohol. At 2°–3°C it crystallizes from water with 47 molecules of crystal water; melting point 46°C. At 20°C it crystallizes with 38 molecules of crystal water; melting point 58–59°C. By heating to 120°C most crystal water disappears, but 3 molecules of crystal water are held so firmly that they only disappear at the destruction temperature.

The calcium salt is a little more easily soluble than hydroxyl apatite[5]).

Phytin forms salts which are dissolved with difficulty or are insoluble, and many different double salts. A salt of special interest is the ferric salt which is insoluble in $^1/_6$N hydrochloric acid and thus can be separated from ferric phosphate. It is precipitated from an acid solution as a slimy whitish yellow precipitate the composition of which depends on the conditions of precipitation[14]). In an excess

of ammonia or amines it forms soluble brown complex compounds. The aluminium salt is also insoluble in $^1/_6$N hydrochloric acid.

Phytin is very resistant to basic hydrolysis and rather resistant to acid hydrolysis. By boiling with acid under pressure it is hydrolyzed through various lower phosphoric acid esters into mesoinositol and phosphoric acid.

Phytin is also rather resistant to oxidation with basic hypobromite [3]).

Phytin is easily dephosphorylated under the influence of the enzyme phytase. P e d e r s e n [11]) found that the optimal activity of this enzyme is at about pH 4.9, and it is inactive below about pH 3.15. Ferric phytate is very resistant to enzymatic hydrolysis [16]) [6]) and calcium phytate is somewhat resistant [6]).

3. ISOLATION AND IDENTIFICATION OF PHYTIN PHOSPHORUS

B o w e r [1]) examined three soils and found that phytin phosphorus and phosphorus in the lower inositol phosphoric acid esters constituted on an average 32.5 and 12.5 per cent respectively of the organically fixed phosphorus in the sodium hydroxide solution.

In order to investigate whether Danish arable land might contain similar quantities of phytin phosphorus, a sample of sandy soil containing a fair amount of humus was taken. The examination of this soil fell naturally into two sections: isolation and identification of phytin and quantitative determination of phytin.

The soil investigated was a diluvial sand from Finderup Skovhuse, which is believed to have been cultivated for about 800 years. The topsoil has a depth of about 50 cm, and there is no hardpan in the subsoil; pH 5.88.

The quantity of organic P was estimated according to a method described by O d y n s k y [9]), although in a slightly modified form: 1 g sifted and pulverized soil is ashed at 600° C. The ashed soil is leached on a filter with 5 × 20 ml $2NH_2SO_4$. The filtrate is made to 200 ml with water. From this solution samples are taken for the determination of P. In a corresponding soil sample P is determined in the same way, but without ashing. The difference between the two determinations is assumed to be organic P.

All determinations of P are made by electrometric colorimetry, a

Coleman photometer being used. The results were as follows:

P soluble in $2N H_2SO_4$ after ashing p.p.m.	574
P soluble in $2N H_2SO_4$ without ashing p.p.m.	220
Organic P.p.p.m.	354

Isolation of phytin was made according to a method similar to that described by B o w e r for quantitative determination of phytin. 2.5 kg soil was leached in a Büchner funnel with 1 per cent. HCl until the presence of Ca^{++} could not be shown in the filtrate. The coarser particles (mainly quartz) were separated by washing and discarded. After the washings had been filtered off with a Büchner funnel, the finer particles (about one third of the original amount of soil) were transferred to a 2000 ml boiling flask and about 1500 ml of 4 per cent. NaOH was added, after which the flask was placed on a water bath for 4 hours at 85–90°C. After boiling the soil sample was filtered off with a folded filter. About 25 ml of bromine was added to the blackish brown filtrate, which was then placed on a water bath at 40–50° for 24 hours. A large yellowish precipitate was formed which was filtered off with a folded filter. Even after having been passed three times through the filter the filtrate did not become clear. The slightly turbid yellowish brown filtrate was acidified with 20 per cent. HCl. The precipitate resulting from this process was filtered off, after which the surplus of bromine was boiled off. After cooling, the solution was adjusted to $\frac{1}{6}$N with regard to hydrogen ion with 20 per cent. HCl, and was then treated with an excess of 5 per cent. $FeCl_3$, which produced a precipitate. In order to flocculate the precipitate, the solution was boiled for about an hour on a water bath. After cooling the precipitate was centrifuged off and washed twice with $\frac{1}{6}$N HCl. The precipitate, which was of an intense brown colour because of impurities, was decomposed with 10 ml of 5 per cent. NaOH, and the ferric hydroxide formed was centrifuged off and washed with 2 × 5 ml of 5 per cent. NaOH. In order to purify the NaOH solution, which was nearly pitch black, it was oxidized with 5 ml of bromine for about 10 minutes at 80°C. A large yellowish precipitate was formed. According to W r e n s h a l l and D y e r[14]) a precipitate which they had obtained in a similar way was ether soluble. This also proved true of the precipitate mentioned above. Solution and precipitate were poured into a separatory funnel and shaken with ether until most of the bromine and the whole of the

precipitate had been removed. Then the sloution was acidified, and a precipitate appeared. This precipitate and the rest of the bromine were removed by shaking with ether and a solution clear as water was obtained. The solution was transferred to a test tube and was precipitated with an equal volume of 96 per cent. alcohol, which caused a flocculated precipitate to form. After 24 hours the precipitate had formed a mass clear as syrup on the bottom of the test tube. The fluid above was decanted off and the precipitate was washed twice by decanting with 5 ml of 50 per cent. alcohol in order to remove the surplus NaOH. The rest of the alcohol was boiled off on a water bath and the precipitate was dissolved in as little water as possible.

According to Pedersen[11]) sodium phytate in an aqueous solution crystallized at 20°C with 38 H_2O after some time. An attempt to make sodium phytate crystallize from the solution mentioned above, however, failed, for on standing for about three weeks an amorphous precipitate appeared, and the supernatant fluid was thick so that it was impossible to separate the precipitate from it. Attempts made with a solution made from very small quantities of pure crystalline sodium phytate gave the same negative result. As sodium phytate crystallizes at 3° with 47 H_2O (see p. 254), an attempt was made to crystallize it at this temperature. The precipitate was dissolved in a little water, and the solution was left to stand in a vacuum desiccator at 3°. After standing for about a fortnight, a rather large, slightly yellow, crystalline precipitate had formed which was filtered off and washed with a few drops of water. In order to remove the yellow impurities the crystals were dissolved in about 5 ml water, and the solution was left to stand for crystallization as described above. After a fortnight some large colourless crystals had developed, which were isolated and washed with a little water.

For comparison sodium phytate was produced from magnesium-calcium phytate as follows: a little magnesium-calcium phytate was dissolved in $\frac{1}{6}$N HCl. The solution was precipitated with $FeCl_3$, the precipitate was centrifuged off and washed with 2 × 10 ml. of $\frac{1}{6}$N HCl, after which it was dissolved in 10 ml of 5 per cent. NaOH. The ferric hydroxide formed was centrifuged off, and the completely clear solution was precipitated with an equal volume of alcohol and a flocculated precipitate was formed. After a time this became

concentrated at the bottom into a clear syrupy precipitate, which completely resembled the precipitate obtained from the soil. The precipitate was prepared for crystallization as described above. After standing for about a fortnight, some large crystals had developed, which were isolated.

Furthermore a little pure crystalline sodium phytate was dissolved in water and left to stand for crystallization in the same way. From this sample it was not possible, however, to crystallize enough sodium phytate to isolate it.

As mentioned above, the two salts behaved similarly as regards precipitation with alcohol in aqueous solution. The salts isolated also completely resembled each other macroscopically, and both of them lost crystal water and pulverized very quickly after being isolated. The melting points of both salts were determined in capillary tubes after being air-dried for a quarter of an hour. The melting point was determined several times for both salts. The result appears from Table I.

TABLE I

	Melting point C° for sample:				
	No. 1	No. 2	No. 3	No. 4	Average
Sodium phytate from soil	48	48	46	49	48
Sodium phytate from Mg-Ca phytate	45	46	46	47	46

Several samples did not melt at all even when heated to 100°C. This was probably due to loss of crystal water (only the salt containing crystal water having a well-defined melting point). The melting point of sodium phytate with 47 molecules of crystal water is stated to be 46°C (see p. 254), and the melting points found are in good agreement with this, moreover the mutual agreement is satisfactory.

Both samples were examined optically about 2 hours after being isolated. The result of this examination was given as follows *):

"Both samples were moist when the examination started, but soon dried after pulverization. Both samples proved to be birefringent under the polarization microscope.

*) The optical examinations were made at the laboratory of "Kryolitselskabet Øresund". My cordial thanks are due to the late Richard Bøgvad, M.Sc. The assistance I received was most valuable.

The average refraction "n" was determined by the immersion method. Because of the apparently unstable character of the material this value can only be given to two decimal places.

	Conditions of Refraction
Sample 1 *) soon after pulverization	$1.46 < n < 1.47$
Sample 1 about half an hour after pulverization	
Partly material with	$1.46 < n < 1.47$
„ „ „	$1.47 < n < 1.48$
Sample 2 *) soon after pulverization	$1.47 < n < 1.48$
Sample 2 about half an hour after pulverization	$1.47 < n < 1.48$

Thus there is little difference between the two samples as regards refraction, and it is seen that at any rate the refracting part of sample 1 is identical with or becomes on standing (probably drying) identical with sample 2 within the limits mentioned".

The crystal form could not be determined as the crystals were unstable in the fluids used.

In both samples and in a sample of pure sodium phytate Na and P were determined quantitatively after being dried at 120°C, at which temperature sodium phytate loses all crystal water except three molecules, which are lost only at destruction temperature. In spite of the fact that only 24 mg salt isolated from soil was available, the analyses were carried through in duplicate.

Sodium Determination

About 10 mg substance was dissolved in 100 ml of water. From this solution 5 ml was taken, to which were added 5 ml of isopropyl

TABLE II

	Percentage of Na		
	1	2	Average
Crystalline Na-phytate **)	26.81	28.95	27.88
Na-phytate derived from Mg-Ca phytate †)	26.84	25.73	26.29
Na-phytate isolated from soil	27.64	26.38	27.01

*) Sample no. 1 derived from Mg-Ca phytate; Sample no. 2 derived from soil.
**) Large crystals isolated from wheat bran.
†) Ordinary commercial product.

alcohol and water up to 25 ml. The sodium content of this solution was determined flame spectrophotometrically with a Beckman spectrophotometer, solutions of sodium oxalate being used as standard solutions.

The results of the analyses appear from Table II.

In sodium phytate with three molecules of water the theoretical content of sodium is 28.298 per cent.

Phosphorus Determination

20 ml was taken from the solution used for determination of sodium. An attempt was made to wet-ash this in micro-Kjeldahl flasks with 2 ml of concentrated sulphuric acid. After destruction the contents of the flasks were transferred quantitatively to 100 ml flasks, and aliquot samples for the determination of phosphorus were taken for analyses. The analysis was made with two duplicates. There were, however, such great differences between the duplicates (50–100 per cent.) that the figures were considered to be of no value. As this was probably due to incomplete destruction, another set of samples was taken in hand and the ashing was carried out with the addition of perchloric acid. The results appear from Table III.

TABLE III

	Percentage of P		
	1	2	Average
Crystalline Na-phytate *)	18.07	18.51	18.29
Na-phytate derived from Mg-Ca phytate †)	17.13	16.29	16.71
Na-phytate isolated from soil	17.66	17.61	17.64

The theoretical content of phosphorus in sodium phytate with three molecules of water is 19.089 per cent.

Summary

The identity of the sodium salt isolated from the soil cannot be considered established by this work, but in all the properties investigated it resembles sodium phytate as follows:

(1) it forms a ferric salt which is insoluble in $\frac{1}{6}$N HCl,
(2) it is precipitated with alcohol as a syrupy, clear precipitate,
(3) its melting point is 48°C (according to the literature 46°C),

*) Large crystals isolated from wheat bran.
†) Ordinary commercial product.

(4) it shows the same conditions of refraction as sodium phytate derived from Mg-Ca phytate, and
(5) it has nearly the same contents of sodium and phosphorus as sodium phytate.

4. QUANTITATIVE DETERMINATION OF PHYTIN PHOSPHORUS

An attempt was made to determine phytin phosphorus in the Finderup soil quantitatively according to Bower's method [1]). The apparatus available, however, made it necessary to work with somewhat smaller quantities of soil than those suggested by Bower. The method, however, is very time-consuming. Filtration of the NaOH solution was difficult. It was difficult to obtain completely clear filtrates after the bromine oxidation, and the ferric precipitate obtained was always very brown with impurities.

A method was developed for quantitative determination of phytin phosphorus which is more easily used. The method, which is a modification of that of Bower's, has not been tested sufficiently, and no doubt it will be possible to improve it on various points. From a single investigation made according to this method it seemed to give reasonable results.

The analytical procedure is as follows:

50 g of sifted and pulverized soil is leached on a Büchner funnel with a solution containing 1 per cent. HCl and 1 per cent. $FeCl_3$ until the presence of Ca^{++} can no more be shown in the filtrate. After washing with the acid the soil is transferred to a 1000 ml flask and heated for two hours with 400 ml of 2 per cent. NaOH at 85–90°C. After cooling 10 ml of bromine is added and the flask is put on a water bath at 45–50° until the fluid above the precipitate is a clear yellowish brown. This oxidation must not be prolonged beyond what is necessary because ferric phytate is not absolutely resistant to it. After cooling the precipitate is filtered off. As presumably the organic matter has in part been completely oxidized and has in part acquired other physical properties, the process of filtration is fairly easy even with an ordinary filter. The precipitate is washed on the filter with as little 2 per cent. NaOH as possible. The filtrate, which as a rule is not quite clear, is acidified with 20 per cent. HCl, and the precipitate thus formed is filtered off, after which the surplus of bromine is boiled off. After cooling the solution, which usually is not quite clear, is adjusted with 20 per cent. HCl so that it is at $\frac{1}{6}$N as

regards hydrogen ion. Adjustment of the solution is made by taking an aliquot of 5 ml and titrating with $\frac{1}{6}$N HCl. After titration the titrated solution is poured back into the boiling flask. Bromo-thymol blue is used as indicator. It turns yellow with acid and therefore does not colour the solution appreciably. Phenolphthalein cannot be used as it is destroyed by the small quantities of bromine found in the solution. The acid solution is precipitated with 5 per cent. $FeCl_3$, after which the precipitate is made to flocculate by boiling for 1–2 hours (often no precipitate develops until after boiling for some time). After cooling the ferric precipitate is centrifuged off and decomposed with 8 ml of 5 per cent. NaOH, and the ferric hydroxide is centrifuged off and washed with 2 × 3 ml of 5 per cent. NaOH. The brown-coloured NaOH solution is transferred quantitatively to a 150 ml Erlenmeyer flask. About 5 ml of bromine is added to it and it is placed on a water bath at 40–50°C until it becomes a transparant yellow colour. The oxidation must not be prolonged beyond what is necessary, as ferric phytate, as stated above, is not absolutely resistant to this oxidation. The oxidized solution is transferred quantitatively to a 100 ml long-necked flask with a ground glass stopper and is shaken with ether. After shaking the ether is removed by means of a pipette and ether is added again. This procedure is continued until all the precipitate is removed (2–3 extractions with 25 ml of ether are generally sufficient), after which the solution is acidified with concentrated HCl, which produces a whitish yellow precipitate. The solution is then shaken with ether again until it is as clear as water.

The clear solution is transferred quantitatively to a 80 ml centrifuge tube, adjusted to $\frac{1}{6}$N as regards hydrogen ion with 20 per cent. HCl and is precipitated with an excess of 5 per cent. $FeCl_3$. The centrifuge tube is then placed on a water bath and the solution is boiled for 1–2 hours. After cooling the almost pure white precipitate formed is centrifuged and washed twice with $\frac{1}{6}$N HCl. The precipitate is then decomposed with 8 ml of 5 per cent. NaOH and the ferric hydroxide formed is centrifuged off and washed with 2 × 3 ml of 5 per cent. NaOH. The solution of sodium hydroxide is transferred quantitatively to a micro-Kjeldahl flask and destroyed with H_2SO_4 and perchloric acid (it is necessary to use perchloric acid as the destruction is rather difficult, cf. p. 260). After the destruction P is determined in the usual way.

It is a condition of the methods, i.e. B o w e r's original method and the modified one described here, that the white ferric precipitate does not contain any other phosphorus compound except phytin so that its contents of phosphorus can be expected to be phytin phosphorus.

The method described has two weak points. It is difficult to avoid loss during the shaking with ether, which possibly might be performed in a better way. Furthermore it is not certain that phytin is precipitated quantitatively by $FeCl_3$ as ferric phytate is apt to form supersaturated solutions and be precipitated slowly. For instance it was discovered that in a solution which had been centrifuged free from a ferric precipitate, a small precipitate of ferric phytate formed on standing for three months. Perhaps the boiling after precipitation with $FeCl_3$ should be prolonged and the solution stand for 24 hours or more before being centrifuged.

In order to test the reliability of the method, analyses were made of 4 samples of the soil from Finderup. Sodium phytate containing 2.95 mg phytin phosphorus was added to samples 3 and 4, while nothing was added to samples 1 and 2. Unfortunately a great part of the content of the Kjeldahl flask was lost during boiling at the destruction of sample 1. The results of the analyses appear from Table IV.

TABLE IV

No.	Phytin phosphorus added mg	Phytin phosphorus in 50 g soil mg	Phytin phosphorus in 50 g soil— phytin phosphorus added mg
1	0	(4.44)	
2	0	8.16	8.16
3	2.95	9.28	6.33
4	2.95	10.92	7.97

It is seen that there is a fairly great difference between the replicates. The phytin added has obviously in part reappeared in the analyses, but the amount added was too small since it only constituted about one third of that contained in the soil, which was surprisingly great.

The distribution of the phosphorus in the Finderup soil is given in Table V, 8.16 mg phosphorus (sample 2) being considered to be the content of phytin phosphorus in 50 g soil.

TABLE V

Distribution of phosphorus in the Finderup soil	
P dissolved in 2N H_2SO_4 after ashing p.p.m.	574
P dissolved in 2N H_2SO_4 without ashing p.p.m.	220
Organic P p.p.m.	354
Phytin phosphorus p.p.m.	163
Org. P per cent. of P diss. in 2N H_2SO_4 after ashing	61.3
Phytin P per cent. of P diss. in 2N H_2SO_4 after ashing	28.4
Phytin P per cent. of organic P	46.0

It is seen that phytin phosphorus constitutes a very large part of the organic phosphorus, and as the method for the determination of organic phosphorus probably gives too high values whilst the determination of phytin phosphorus rather gives too low values, phytin phosphorus no doubt constitutes a still greater part of the organic phosphorus than shown by the analysis. In relation to the total content of phosphorus in the soil also, phytin phosphorus constitutes a very considerable part since it must be supposed that the main part of the total phosphorus can be extracted with 2N H_2SO_4 after ashing.

5. PHYTIN PHOSPHORUS IN THE SOIL

Thus it seems that considerable amounts of phytin phosphorus may occur in the soil. It is not known for certain from where the phytin phosphorus originates. Presumably it must originate from dead vegetable matter, be supplied in organic manure, or be built up by micro-organisms. Unfortunately few investigations into the distribution of phytin in nature have been published. What have been investigated best are the grain of the cereals and oil-cakes. In grain phytin phosphorus constitutes 60–80 per cent. of total phosphorus and in oil-cakes about 80 per cent [11]). Hart and Tottingham [4]) found that swedes and lucerne hay did not contain phytin phosphorus.

Kaila [6]) [7]) determined acid soluble organic phosphorus in various materials after a preliminary extraction with alcohol and water. Kaila assumed that acid soluble organic phosphorus was mainly phytin phosphorus, which at any rate presupposes that no iron is present, for if it were, phytin would be precipitated as ferric phytate. Kaila found that in fresh manure 5–14 per cent. of phosphorus was found as acid soluble organic phosphorus, and in

poultry dung as much as 39 per cent. When the manure was stored, part of the acid soluble organic phosphorus was mineralized. The composition of the organic phosphoric compounds of the manure depended on the kind of compounds found in the fodder. In straw, hay, potatoes, peas, and moss, the content of acid soluble organic phosphorus was comparatively low while an Actinomyces species had a high content.

Hence the content of phytin in the vegetative parts of plants is presumably not high, but as cereals and other seed crops can hardly be removed completely from fields some phytin must be supplied to the soil in the course of time. The accumulation of the large amounts of phytin found in soil must take place by the phytin being held in compounds which are mineralized with difficulty. Wrenshall, Dyer, and Smith [15]) found that phytin added to a soil was not mineralized. As mentioned above, ferric phytate is very resistant to enzymatic hydrolyses, and calcium phytate is rather resistant. This makes it probable that phytin in acid soils occurs as ferric phytate, and there is some probability that phytin in basic soils occurs as calcium phytate (basic soils have never been investigated for phytin).

Investigations by Pearson, Norman and Chung Ho [10]) showed that phytin added to Ames fine sandy loam with pH 5.8 was mineralized very slowly, but if $Ca(OH)_2$ was added, so that pH increased to 7.2, the mineralization occurred more quickly, but it did not exceed 40 per cent. in 90 days.

Culture experiments with phytin salts all show that phytin can actually be utilized by the plants but that generally it has a poorer effect than inorganic phosphates, and that the effect greatly depends on the type of soil [13]) [8]) [12]) [2]). It should be noted that Saurlandt [12]) found that in a sandy soil where the effect of the addition of phytin was marked, the effect decreased considerably when $CaCO_3$ was added.

SUMMARY

Phytin was isolated from a diluvial sand which has been cultivated for about 800 years. Various investigations of the organic phosphoric compounds isolated made it probable that the substance was phytin.

A method for a quantitative determination of phytin phosphorus in soil was worked out. In the heath soil in question 163 p.p.m. phytin phosphorus

was found to correspond to 46 per cent. of the total content of organically held phosphorus.

In acid soils phytin is presumably found as ferric phytate and in basic soils possibly as calcium phytate.

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CONTROL OF THE REPRODUCIBILITY AND ACCURACY OF ROUTINE ANALYSES AT THE LABORATORY FOR SOIL AND CROP TESTING IN THE NETHERLANDS

by F. H. B. VERMEULEN

Agronomist to the Laboratory

The laboratory for soil and crop testing in the Netherlands, with its branches at Oosterbeek, Groningen, Geldrop and Goes, carries out analyses of soils and crops for farmers and growers. Based on the results of these analyses the Advisory Service advises farmers on manuring and feeding.

On account of the large number of samples to be examined (about two million analyses per year) the analytical methods have been carefully standardised and made as simple as possible so that they can be carried out by girls having no previous experience of chemical work. These laboratory workers are trained at the laboratory and work under the supervision of experienced analysts.

The advantages of using non-technical labour and the high operative speed resulting from the routine acquired must be offset against the need for an extensive system of controls to check the accuracy of the work. In order that the analyses carried out at the various laboratories shall be comparable, mathematical treatment of the analytical results from each laboratory and analyses of standard samples by all the laboratories are necessary. Such procedures make it possible to obtain some idea of the difficulties involved in routine analyses and the reliability of the results.

METHODS OF CONTROL

A. Standardisation of glassware, chemicals etc.

B. Determination of the reproducibility of the analytical data.

C. Determination of the accuracy of the analyses.

A. Random samples from each new consignment of glassware, chemicals or filter paper are analysed in order to ascertain whether

they comply with the standard requirements. Whether the glass will release ions which would interfere with the analyses must always be determined. The calibration of glassware (volumetric flasks, pipettes, thermometers etc.) and the standardisation of extraction fluids and reagents is carried out by analysts.

B. It is essential that the analytical results are of a suitable order of accuracy. To ensure this the analyses are checked against standard methods by which the composition of various materials can be determined with great accuracy. All series of samples of material issued to any laboratory worker for analysis include a solution of known composition; the results obtained for the test sample should of course agree with the known value.

C. Since all analyses are duplicated the reproducibility of the analyses can be ascertained by calculation of the standard deviation.

Control of reproducibility of the analyses

One or more standard samples are always added to each series of samples being analysed. The material of the standard sample is always thoroughly homogenised and hence the analytical results which should be obtained are known. In the case of determinations of ions a solution containing known concentrations of particular ions is added to the series. This procedure enables inaccuracies to be detected at once. Once a month the reproducibility of results among all four laboratories is checked by distributing a number of identical standard samples among these laboratories and the analytical results compared at a central laboratory. This method of control allows inaccuracies occurring in any of the laboratories, and which might not be detected by local control, to be detected. Such inaccuracies may occur for example if a faulty extraction temperature is being generally used at one laboratory.

As already stated the reliability of the analyses can be judged from the standard deviation. This may be calculated (*a*) from the analyses of the standard samples according to the formula

$$s = \sqrt{\frac{\Sigma d^2}{n - 1}}$$

where d represents the deviation of the individual values from the sample mean and n the total number of sample values, or (*b*) from the duplicate determinations on the field samples according to the

formula

$$s = \sqrt{\frac{\Sigma y^2}{2n}}$$

where y represents the difference between the duplicate determinations and n the number of field samples (i.e. number of analyses $= 2n$).

It has been found that for all determinations there is an approximately quadratic relationship between the standard deviation and the actual content of a given constituent, the standard deviation increasing as the content increases. The standard deviation, expressed as a proportion of the content of a given constituent, decreases, however, with increasing content of the constituent.

A comparison of the reliability of any two sets of determinations can therefore be made only by direct comparison of the standard deviations if the mean contents of the two sets are the same. When this is not the case, valid comparisons can only be made when the two sets of data are divided into a number of corresponding groups with approximately the same standard deviations. The objection to this method is that it requires a large number of determinations.

Nor is it possible to compare monthly calculations of the standard error from the accumulated data since the mean content of a constituent, and hence the standard deviation, may vary from month to month even though the accuracy remained the same. In such cases the data would again have to be split up into groups.

The standard deviation may be considered as consisting of two components, one representing the actual experimental error which occurs even when blank determinations are carried out. This analytical error is not related to the value determined. The other component of the standard deviation arises from faults in sampling the test material and these will obviously have an influence on the values obtained. The standard deviation consists therefore of a constant and also a variable component.

The relationship between standard deviation and the phosphate status of the soil as determined by the analysis of a 1% citric acid extract using the molybdenum blue method is depicted in Fig. 1. Phosphate contents are plotted on the x axis and standard deviation calculated for groups on the y axis. In this particular case the relationship may be expressed by the equation:

$$s = \sqrt{1.61 + 0.00157\, x^2}$$

Objections to this method of calculation of the standard deviation

for groups (the amount of mathematical work involved and the need for a large number of determinations) led W. C. Visser, sometime agronomist to the Agricultural Experimental Research Station at Groningen, to modify the method of expressing the results whereby a measure for the standard deviation is obtained which is independent of the phosphate content. The appropriate transformation was $Q = \log (x + \sqrt{x^2 + 1025})$ where x represents the phosphate content determined by the particular analysis.

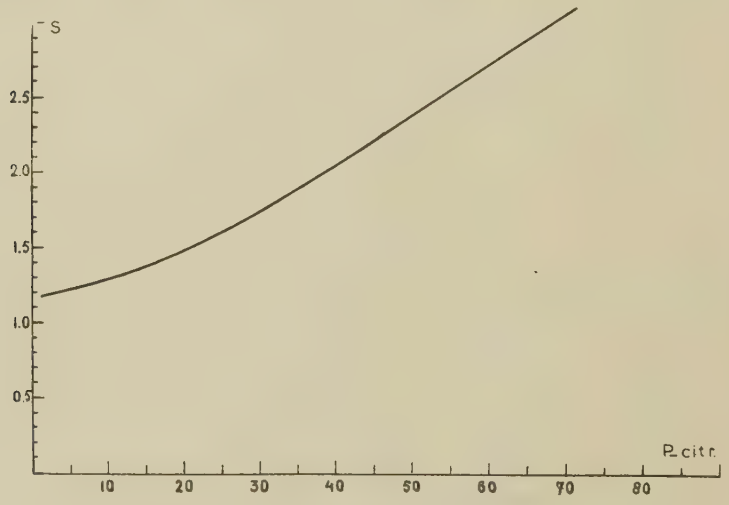

Fig. 1. Relationship between P_2O_5 content of the soil and the standard deviation.

For any value of duplicate phosphate determinations, the corresponding values for Q may be calculated according to the above equation and the difference between these Q values may be used to calculate the standard deviation as before but with the advantage that the mean contents need not now be the same for a valid comparison of the results to be made. To avoid the necessity for calculating the two Q values for each duplicate determination Fig. 2 has been prepared giving, along the ordinate, the differences between the two Q values corresponding to differences of 1, 2, 3, 4 etc. between the two observed phosphate contents over a wide range of mean phosphate contents.

Subsequently as a check the duplicate differences were plotted on the curved lines in a diagram as represented by Fig. 2, after which the

standard deviations were calculated for different groups of phosphate contents. The standard deviations were obtained according to the formula

$$s = \sqrt{\frac{\Sigma v^2}{2n}}$$

where v represents the linear distances from the different points in the diagram to the abscissa. The values obtained for s are represented by the dotted line in Fig. 2. It is to be noted that the standard deviation is appreciably independent of the phosphate content.

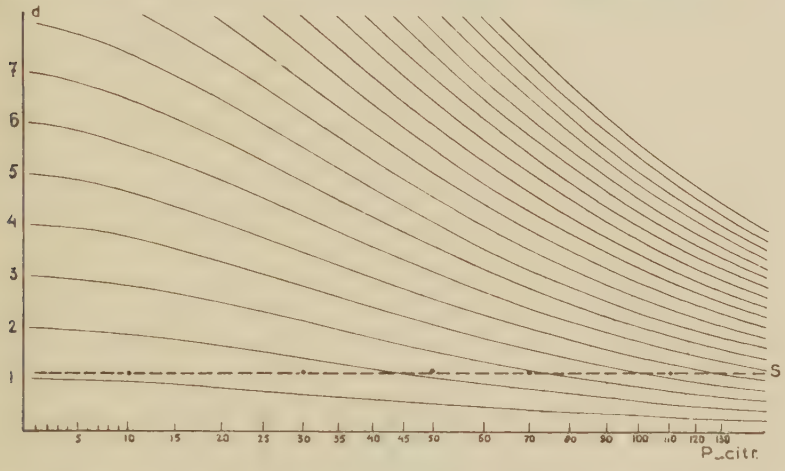

Fig. 2. Diagram for the determination of the P_2O_5 content of the soil (constant standard deviation).

Duplicate differences obtained at high phosphate contents can now be compared directly with duplicate differences at low phosphate contents thus enabling control of the analytical results to be maintained with far less data. In addition the absolute standard deviations associated with any phosphate content can be read off from the diagram directly and the relative standard deviation (as percentages) can be readily calculated from these.

By checking all parts of the analytical procedure it is possible to attain a very high degree of accuracy such as is desirable from a chemical point of view. From the point of view of the agronomist it is not at all certain whether such reduction of analytical errors is necessary in view of the relatively large sampling errors. The following examples show however that in routine soil analyses careful

control is essential. In particular, certain aspects which until a few years ago could not be checked will receive particular attention.

1. *The data from which the standard error is calculated must agree with the frequency distribution curve*

Fig. 3 represents the frequency distribution of the values obtained in the determination of the K-status of the standard soil samples. (In determinations of the K-status of sandy soils the content of adsorptive material, e.g. humus, is taken into account). The dotted curve represents the standard distribution calculated from the standard deviation.

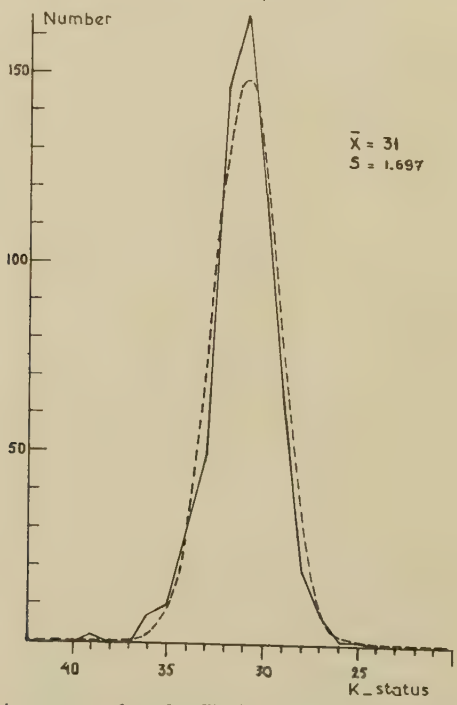

Fig. 3. Distribution curve for the K_2O determinations for a standard soil sample. The normal distribution curve is indicated by the dotted curve.

Since there is very good agreement between the experimental and the calculated curves, the calculated curve for the standard deviation can be used as a basis for the control curve for K determinations.

In the same way all determinations have been checked to ensure that the data have the correct frequency distribution.

2. *The effect of shaking on the reaction, in terms of pH units, of the soil-water suspensions*

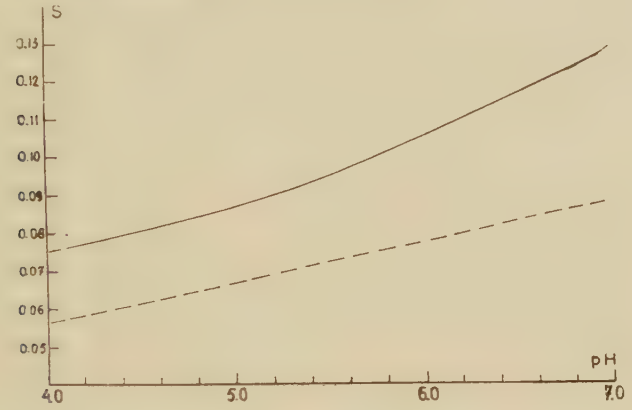

Fig. 4. Relation between reaction (pH) and standard deviation. Values for samples shaken three times indicated by the dotted curve; values for samples shaken twice by the continuous curve.

The reaction of soil suspensions has been determined by the quinhydrone method. The effect on such measurements of shaking the soil suspensions two or three times by hand was determined. The results (Fig. 4) indicate that the standard deviation is appreciably higher when the suspension is shaken twice than when it is shaken three times. Investigations showed that increased amounts of shaking did not improve the reproducibility of the results.

3. *The effect of the shape of the glassware used for extraction*

In determinations of the P-status (the amount of phosphate soluble in water at 50°C) a known amount of soil is added to a known volume of water and the mixture allowed to stand for 24 hours at 50°C. The extract is then separated by filtration and the phosphate content determined by the molybdenum blue method.

Since it is desirable, if possible, to use thick-walled glassware in order to minimise breakages the effect of the shape of the glassware used for extraction was investigated. In a comparison of extraction in (*a*) cream bottles and (*b*) Erlenmeyer flasks, the latter was found to give a much higher degree of reproducibility (Fig. 5). The area of contact between the water layer and the soil layer was larger in the case of the Erlenmeyer flasks (22.9 sq.cm) than in the cream bottles (15.2 sq.cm). If instead of standing upright in the thermostat the

cream bottles were placed on their sides (*c*) the area of contact was increased to 22.4 sq.cm. By this method the reproducibility of the P-status determinations was improved to the same level as that obtained by the use of Erlenmeyer flasks (Fig. 5).

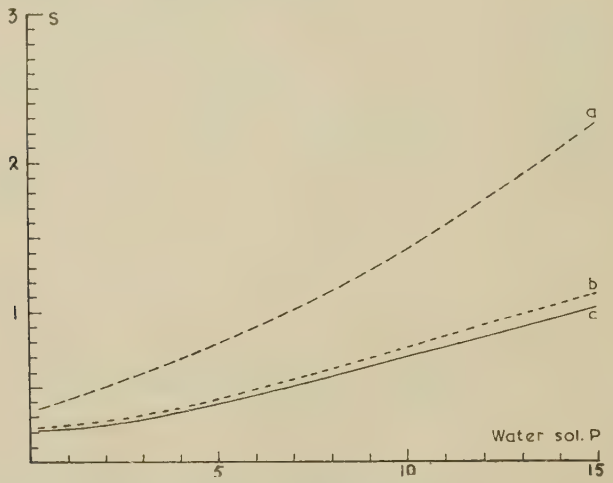

Fig. 5. Influence of the shape of the glassware used for extraction.
(*a*) cream bottles in upright position.
(*b*) Erlenmeyer flasks.
(*c*) cream bottles in horizontal position.

4. *The effect of season and casual occurrences on the reproducibility of the results*

As well as the direct effect, which can be avoided by taking the necessary precautions, of factors such as temperature during extraction, air pressure and air humidity on the various analytical procedures, the working conditions are also subject to indirect influences which may be greater at one time than at another. It is useful to know when and under what conditions the accuracy of the work suffers as it is then possible to determine when extra attention by the supervisory staff is necessary.

As an example, the determination of soil organic matter by loss on ignition was observed over a period of about one year. In this method the amount of organic matter in the soil is calculated from the loss in weight on ignition of the soil at a temperature of 900°C.

Fig. 6 represents the standard deviations calculated over the period June 1950–June 1951 and high peaks represent a high degree of

error. A consideration of every peak would necessitate too long a discussion, only the more important points will be dealt with.

Experience has shown that after the summer holiday season, with the coming of autumn, the staff work with greater concentration; this is borne out by the regular fall in the standard deviation during September 1950. In addition this month is the time when large

Fig. 6. Influence of the season on the reproducibility of the humus determinations.

numbers of samples arrive at the laboratory and work in the laboratory is at a peak. During October signs of fatigue appear and the standard deviation increases, but towards the end of the month there is an improvement.

From November 1950 onwards a gradual increase in the standard deviation will be observed. This increase reaches a peak during the week of Santa Claus (December 6) when work evidently suffers on account of decreased concentration. After Christmas there is a gradual increase in the accuracy of the analyses.

The marked fall in the accuracy of the analyses during the period April 10th to 16th 1951 is due to the coming of spring.

The difference between the standard error for June 1950 and that for June 1951 may be attributed to improvements in the analytical methods introduced during 1951.

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THE MICRO-ORGANISMS IN THE RHIZOSPHERE OF BANANA PLANTS IN RELATION TO SUSCEPTIBILITY OR RESISTANCE TO PANAMA DISEASE

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INTRODUCTION

Why certain banana varieties are extremely susceptible to infection by *Fusarium oxysporum-cubense*, the fungus which causes the destructive Panama Disease, whereas others are highly resistant, has not yet been satisfactorily explained. W a r d l a w [5]) came to the conclusion that, after an initial soil borne infection of the root base has taken place, fungal invasion is checked by the development of protective suberized cambiform barriers, the secretion of wound gums, vessel collapse, and the obstruction of vessel cavities by tyloses. However, no difference in the capacity to form such barriers was observed which could have explained a difference in susceptibility to the disease. It seemed, therefore, that such differences could only arise from variations in virulence or in degree of the initial root base infections, for which the reasons remained obscure.

M e r e d i t h [4]) isolated various Actinomycetes, which were able to inhibit or retard the development of *F. oxysporum-cubense*, from Jamaican soils where Panama Disease did not occur to any serious extent. Although this observation did not elucidate the specific susceptibility of certain banana varieties, they had the merit of stimulating the study of the possible influence of associated soil micro-organisms on the rate of infection by *F. oxysporum-cubense*.

H a r p e r [1]) started from the sound assumption that, if such influences existed in connection with specific resistance against the primary root base infection, they most probably had to be looked for

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in the rhizosphere of the banana plants. He obtained very encouraging results. A bacterium, strongly antagonistic to *F. oxysporum-cubense* was isolated in high numbers from the rhizosphere of the Panama Disease resistant Congo banana, but was found to be virtually absent from the genetically allied Gros Michel banana, known to be very susceptible to the disease.

H a r p e r pointed out that the limited number of his observations made it desirable to carry out further investigations to determine as to whether the difference in distribution of his fungistatic organism was a permanent character of certain banana varieties and correlated with their resistance to Panama Disease.

EXPERIMENTAL

In these experiments, with one exception (experiment 7), only two varieties of *Musa acuminata* were used: the highly susceptible Gros Michel, and the virtually immune Congo.

For quantitative analyses of the rhizosphere microflora it is difficult to find means of expressing the figures in such a way that a comparison of results is justified. This holds particularly in the case of the banana plant, which produces its roots from a solid roundish rhizome. Two extreme types of roots may be observed: the one a smooth fleshy root, showing only a few, if any, secondary rootlets; the other a more fibrous root and bearing masses of rootlets. This second type is the fully developed root form, whereas the first type is a juvenile form. Therefore all intermediate forms between these two extremes can be found, but the rate at which the secundary rootlets are formed depends on many factors.

In order to obtain a picture of the rhizosphere microflora we have restricted ourselves to the determination by means of counting plates of the Rhizosphere: Soil ratio (R/S), that is the number of organisms in the rhizosphere soil divided by the number present in the soil at a distance from the roots, both calculated on a dry weight basis. This method of expression has been used following L o c h h e a d [3]) rather than the method used by H a r p e r [1]) in which the weight of root supporting the rhizosphere population is taken into consideration. The estimation of the R/S ratio is less cumbersome and was capable of giving all information required for this discussion. The R/S ratio was always found to be greater than 1 as would be ex-

pected, and confirms Harper's observations on bananas. The figure itself has little significance, since it varies considerably with the nature of the rhizosphere soil which is subjected to the influence of too many unknown factors. However, if *in the same sample* the microflora of the rhizosphere and of the remote soil is divided into certain groups (fungi, actinomycetes, bacilli, etc.) and the R/S for each of these groups estimated, a comparison of these ratios with the R/S of the total microflora brings to light any specific encouraging or depression of particular groups of micro-organisms under the influence of banana roots.

Experiment 1. The distribution of different groups of micro-organisms in the rhizosphere.

Roots were cut off from the rhizomes and the excess of soil removed by gentle shaking. The roots with attached soil were hereupon shaken for 10 minutes with 10 times their weight of sterile Ringer's solution made up to $^1/_{10}$ normal strenght. After shaking the larger particles were allowed to settle for 5 minutes and dilutions of 10^5, 10^6, and 10^7 made of the supernatant suspension. From these dilutions portions of 0.6, 1.0 and 2.0 ccm were pipetted in six fold into Petri dishes and soil extract agar, prepared as described by Harper, added. Where soil out of the influence of roots was to be analysed, the plates were made up from dilutions 10^4, 10^5, and 10^6. The plates were left at room temperature (25°C) for two days after which they were examined. The R/S of the total microflora was calculated from counting all visible colonies in some of the plates.

Subsequently about 100 colonies were taken from rhizosphere plates and transferred to slides for microscopic examination. In order to avoid any preferential selection every single colony was examined of two or more plates on which not more than 50 colonies had developed. On microscopic examination they were classified into four groups: fungi, actinomycetes, sporeforming bacilli, and "others". The proportional occurrence of each group was expressed in percentages from which their R/S was calculated with the aid of the R/S of the total microflora. This was less elaborate than estimating the R/S of every group by means of counting plates. The enormous differences in R/S of the total microflora between each sample are most likely to result from the simultaneous influence of the many factors outlined above. The figures are listed in Table I.

TABLE I

Distribution of the main groups of micro-organisms in the rhizosphere as compared with the remote soil

Site	Nature of Sample	*R/S* of total micro-flora	Fungi		Actino-mycetes		Spore forming Bacilli		Others	
			%	*R/S*	%	*R/S*	%	*R/S*	%	*R/S*
Grounds	Congo rhizosph.	7	2	3.5	14	2.9	6	1.7	78	25.6
C.M.R.I.	G. Michel ,,	26	3	19.5	22	16.8	17	17.7	58	40.8
(No P.D.)	Remote Soil	—	4	—	34	—	25	—	37	—
Collection	Congo rhizosph.	4	2	4	28	4.7	17	2.6	53	4.7
I.C.T.A.	G. Michel ,,	5	2	5	2	0.4	13	2.2	83	9.2
(+ P.D.)	Remote Soil	—	2	—	24	—	29	—	45	—
Collection	Congo rhizosph.	11	3	83	24	8.3	17	5.1	56	19.3
I.C.T.A.	G. Michel ,,	92	5	115	24	69	22	63.3	49	140.9
(+ P.D.)	Remote Soil	—	4	—	32	—	32	—	32	—
Collection	Congo rhizosph.	70	1	23.3	2	4.8	8	12.0	89	249.2
I.C.T.A.	G. Michel ,,	33	1	11	4	4.6	11	8.4	84	110.9
(+ P.D.)	Remote Soil	—	3	—	29	—	43	—	25	—

C.M.R.I. = Colonial Microbiological Research Institute.
I.C.T.A. = Imperial College of Tropical Agriculture.
P.D. = Panama Disease.

Experiment 2. The distribution of fungistatic organisms in the rhizosphere.

Some of the rhizosphere plates, prepared as indicated above (exp. 1), were covered with glucose-marmite-peptone agar, heavily seeded with conidia of *F. oxysporum-cubense*. This was done after the colonies had grown for 5 days and gave, when the Fusarium had been allowed to grow, a quick impression of the number of micro-organisms that had been able to produce in that length of time a sufficient amount of fungistatic antibiotic to show an inhibition zone in the fungus growing over them. With the exception of one sporebearing bacillus and a few types of Pseudomonas the only activity which could be detected by this technique was produced by Actinomycetes, of which about 8% showed a smaller or larger ring of inhibition of the Fusarium. They were found to be present in the same proportions in all samples and there was no difference in distribution between the two varieties of bananas.

A further test was therefore made in the way which led H a r p e r to the discovery of his active Bacterium. About 120 colonies were transferred from both Congo and Gros Michel rhizosphere plates

into tubes with 1% yeast extract broth, using for this purpose every single colony from plates which showed under 50 colonies per plate, in order to avoid preferential sampling. After 10 days the broth was tested for antibiotic activity. About 0.1 ccm was transferred by means of a sterile pipette into a hole of 1 cm diameter made with a cork borer in agar plates seeded with conidia of *F. oxysporum-cubense*. After incubation at room temperature a zone of inhibition of the fungus could be noticed in cases of a positive antibiotic effect. The results of these experiments are listed in Table II.

TABLE II

The distribution of fungistatic organisms in the rhizosphere of Gros Michel and Congo

Site	Nature of sample	Number of colonies tested	Number of fungistatic organisms	Number of fungistatic organisms as would be expected if Harper's observations were applicable
C.M.R.I. (no P.D.)	Congo rhizosphere	120	1 Actinomyc.	2 Bact. plus Actinomyc.
	G. Michel rhizosphere	126	2 Actinomyc. 1 Pseudomon.	24 Bact. plus Actinomyc.
	Remote Soil	122	1 Bacillus 3 Actinomyc.	—
Collection I.C.T.A. (+ P.D.)	Congo rhizosphere	133	1 Pseudomon. 3 Actinomyc.	2 Bact. plus Actinomyc.
	G. Michel rhizosphere	110	1 Pseudomon.	21 Bact. plus Actinomyc.
	Remote Soil	121	2 Actinomyc.	—
Ditto (other banana stools)	Congo rhizosphere	120	1 Actinomyc.	2 Bact. plus Actinomyc.
	G. Michel rhizosphere	116	2 Actinomyc.	23 Bact. plus Actinomyc.
	Remote Soil	121	2 Actinomyc.	—

From Table II it is clear that, in the material used by us from the I.C.T.A. plot, there was no distribution of active bacteria in the manner observed by Harper.

Experiment 3. The influence of rhizome extracts.

The experiments, described above, were repeated in a slightly different way. Watery rhizome extracts of both Gros Michel and

Congo were prepared as described by H a r p e r. These extracts were partly sterilized with 2% agar, and partly as a liquid medium in tubes. Samples of rhizosphere soil of Gros Michel and Congo were plated out with their own rhizome extract agar and after 5 days of growth at room temperature about 240 colonies of both banana varieties were selected non-preferentially. 120 of these colonies were transferred into tubes with Gros Michel rhizome extract broth, and the remaining 120 into tubes with Congo rhizome extract broth. In the broth they were left to grow for another 10 days, after which time they were tested for activity by means of both Congo rhizome extract agar and Gros Michel rhizome extract agar seeded with *F. oxysporum-cubense*. Holes were made in the agar with the aid of a 1 cm cork borer and about 0.1 ccm of the broth were dropped in these holes from a sterile pipette. The results of these experiments are listed in Table III.

TABLE III

Number of fungistatic micro-organisms amongst 120 isolated from the rhizosphere when grown and tested under the influence of rhizome extracts

Rhizosphere of:	Micro-organisms grown in extract broth of: Gros Michel rhizome		Congo rhizome	
	Tested on rhizome extract agar of Gros Michel	Tested on rhizome extract agar of Congo	Tested on rhizome extr. agar of Gros Michel	Tested on rhizome extr. agar of Congo
Gros Michel	1 Actinomyces	3 Actinomycetes 1 Pseudomonas	2 Actinomycetes 1 Pseudomonas	None
Congo	None	1 Actinomyces	2 Actinomycetes 1 Bacillus	1 Pseudomonas

Experiment 4. Newly formed microflora of the rhizosphere.

Rhizome of both Gros Michel and Congo were deprived of all their roots after the pseudostem had been trimmed so that a stump of about 10" was left. The rhizomes were thereupon cleaned in running water with the aid of a hard brush and soap, and subsequently the outer layer was peeled off with a knife. The clean rhizomes were left submerged for 10 minutes in a 0.25% solution of "Aretan", and this was followed by a prolonged rinsing in running tap water. The rhizomes, which after this treatment lodged only a small number of micro-organisms, were then planted in large clay flowerpots, filled with nursery soil made up from equal parts of river sand and compost. Two pots were planted with Gros Michel, two pots with Congo,

and two others were left as a control. After 8 weeks of active growth, during which time no weeds had been allowed to grow in the flower-pots, the banana plants were removed and the microflora of the soil attached to the newly developed roots was analysed. The *R/S* ratio's of the different groups of micro-organisms are presented in Table IV. These group ratio's are not essentially different from those obtained from the fields as recorded in Table I, since here again an encouraging effect is only seen in the group referred to as "others".

TABLE IV

Groups of micro-organisms in newly formed rhizosphere expressed as percentages of the total microflora.

Nature of sample	*R/S* of total microflora	Fungi		Actinomyc.		Spore bearing Bacilli		Others	
		%	*R/S*	%	*R/S*	%	*R/S*	%	*R/S*
G. Michel 1	4.3	2	2.9	12	1.3	10	2.7	76	8.2
G. Michel 2	4.5	2	3.0	30	3.3	11	3.1	57	6.4
Congo 1	7.5	1	2.5	12	2.2	9	4.2	78	14.6
Congo 2	5.1	2	3.4	19	2.4	10	3.2	69	8.8
Controls (average)	—	3	—	41	—	16	—	40	—

For the detection of micro-organisms, capable of producing fungistatic substances, the rhizosphere soil was plated out with soil extract agar and 200 colonies were checked on their activity against *F. oxysporum-cubense*. From the Congo rhizosphere 12 colonies of bacteria were found active, from the rhizosphere of Gros Michel 8. All these 20 colonies consisted of apparently one single species of a yellow Bacterium, which could be classified as a *Pseudomonas*.

Experiment 5. The influence of different soil types on the composition of the rhizosphere microflora.

Rhizomes of both Congo and Gros Michel were cleaned and partially sterilized as directed under experiment 4. One rhizome of each variety was planted at the San Juan Estate in so called "Chocolate Soil", developing over glauconite limestone in the hills of the Central Range in Trinidad. This soil is considered to be of the highest quality found in the island, and enables the cultivation of Gros Michel practically without Panama Disease. A second rhizome of both varieties was planted in the Banana Collection plot of the

Imperial College of Tropical Agriculture at the spot where, shortly before, 3 Gros Michel stools had been completely destroyed by Panama Disease. All four banana plants were allowed to grow for 10 weeks after which time the rhizosphere of the newly developed roots was analysed with the aid of soil extract agar. The results are listed in the Tables V and VI.

TABLE V

Distribution of the main groups of micro-organisms in newly developed rhizosphere in two extreme soil types (field exper.)

Site	Nature of sample	*R/S* of total micro-flora	Fungi		Actino-mycetes		Spore forming Bacilli		Others	
			%	*R/S*	%	*R/S*	%	*R/S*	%	*R/S*
Collection	Congo rhizosph.	24	3	24	14	12.4	15	10	68	48
I.C.T.A.	G. Michel „	48	2	32	12	32	19	25.3	67	94.6
(+ P.D.)	Remote Soil	—	3	—	27	—	36	—	34	—
San Juan	Congo rhizosph.	12	8	32	9	5.1	18	5.3	65	22.3
Estate	G. Michel „	60	1	20	16	45.7	29	42.4	54	92.6
(no P.D.)	Remote Soil	—	3	—	21	—	41	—	35	—

TABLE VI

Fungistatic micro-organisms present in the newly developed rhizosphere flora in two extreme soil types (field experiments)

Site of experiment	Nature of sample	Number of colonies tested	Number of fungistatic organisms	Number of fungistatic organisms as would be expected if Harper's observations were applicable
Collection I.C.T.A.	Congo rhizosphere	122	1 Actinomyc. 2 Actinomyc.	24 Bact. plus Actinomyc.
	G. Michel rhizosphere	108	1 Pseudomon.	2 Bact. plus Actinomyc.
	Remote Soil	120	2 Actinomyc. 1 Bacillus 2 Pseudomon.	
Central Range (S. Juan Estate)	Congo rhizosphere	118	2 Actinomyc.	23 Bact. plus Actinomyc.
	G. Michel rhizosphere	109	3 Actinomyc. 1 Pseudomon.	2 Bact. plus Actinomyc.
	Remote Soil	114	3 Actinomyc. 1 Bacillus 1 Pseudomon.	—

Here again the general picture is not markedly different from that presented in Table I, which shows a preferential stimulation in the

rhizosphere of the Bacteria referred to as "others". Nothing can be concluded from Table VI which could either explain the differences in the severity in Panama Disease as experienced in practice with the two soil types, or extend Harper's observations on the distribution of fungistatic organisms in the rhizosphere of the two banana varieties into other soil conditions than from which his material was collected.

Experiment 6. Survival of conidia of Fusarium oxysporum-cubense in the rhizosphere.

Soil was taken from between the roots of Gros Michel plants badly attacked by Panama Disease, and another soil sample was collected from between the roots of Congo plants grown in the same plot (banana Collection I.C.T.A.), but showing no symptoms of the disease. The soils were sifted through a 3 mm wire mesh, subsequently heavily seeded with a suspension of conidia of *F. oxysporum-cubense* in water, and each soil sample thoroughly mixed with the conidia. Six sterile flower pots were filled with the Congo soil plus *Fusarium conidia*, and six others with the Gros Michel soil plus *Fusarium conidia*. The pots were thereupon buried in a shady place so that the rim of the pots was at the same level as the surface of the surrounding soil.

Samples were taken from each pot at intervals and examined for the numbers of *Fusaria conidia* that had survived, all calculated on a dry weight basis. For this purpose dilutions of the soil suspension in $^1/_{10}$ Ringer's solution were plated with a selective medium for the growth of fungi. This medium was made up as follows: 4% glucose, 0.3% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4$, 0.1% KH_2PO_4, trace of $FeCl_3$, enough HCl to bring the pH at 3.5, and 3% agar. The results are listed in Table VII.

As seen from the table there is very little difference in the rate of decrease in number of Fusaria in the two soil samples.

The experiment was repeated in a somewhat different way. Rhizosphere soil of both Gros Michel attacked by Panama Disease, and Congo was weighed off in portions of 25 g, and each portion transferred to a large sterile testtube (1″ × 7″). 20 ccm of a heavy suspension of *Fusarium conidia* in water were added to each testtube which was thereupon vigorously shaked. Six similar tubes were

TABLE VII

Survival of Fusaria in Gros Michel and in Congo rhizosphere soil in flower pots						
Date of Sampling	Number of Fusaria per gram dry soil		Percentage of Fusaria survived since the start of the experiment		Number of other fungi per gram dry soil	
	G. Michel	Congo	G. Michel	Congo	G. Michel	Congo
20/11 (start)	1500	1400	100	100	7200	5800
27/11	1300	1270	86.7	90.7	5700	5200
4/12	510	420	34.0	30.0	4800	3300
11/12	175	130	11.7	9.3	6100	6700
18/12	159	85	10.6	6.1	7800	8400

prepared in this way of both banana varieties and left at room temperature. Every week the tubes were again heavily shaken and 1 ccm portions taken from each tube for plating onto the selective medium to estimate the number of Fusaria still present. The results are recorded in Table VIII.

TABLE VIII

Number of fungi surviving under the influence of rhizosphere soil (Average of six testtube trials)				
Date of assay	Congo rhizosphere soil		Gros Michel rhizosphere soil	
	Fusaria	Other fungi	Fusaria	Other fungi
23/2 (start)	24600	6800	24600	6600
2/3	28000	7500	28000	6700
9/3	21000	6400	22000	6100
16/3	1900	3400	2700	3600
23/3	130	400	120	500
The figures give the numbers found present in 1 ccm of the soil-water suspension.				

Apart from *F. oxysporum-cubense* being apparently more susceptible to the unfavourable living conditions in the water logged soil samples than some of the saprophytic soil fungi, nothing can be concluded from this table which would indicate any specific influence of the banana roots originally grown in the soil.

Experiment 7. Distribution of fungistatic bacteria in banana rhizospheres.

Forty different banana suckers were examined on the occurrence of fungistatic bacteria in their rhizosphere. These suckers were collected from two fields of the I.C.T.A. The one, field B, was planted

with both Gros Michel and other varieties, the former showing in many cases severe symptoms of Panama Disease. In the other, field 14A, no varieties known as very susceptible to Panama Disease were grown.

From each banana sucker the soil attached to the roots was plated out onto soil extract agar. After 10 days of growth at room temperature plates showing between 20 and 50 colonies were selected for the test. Colonies which had developed on top of the agar were dug out with some of the surrounding agar and discarded. The plates were thereupon flooded with glucose-marmite-peptone agar in which conidia of *F. oxysporum-cubense* were suspended. The removal of the colonies showing surface growth avoided spreading of their bacteria over the plates by the liquid Fusarium agar. Zones of inhibition of the growth of the Fusarium were observed in several cases as can be seen from Table IX.

DISCUSSION

As already mentioned the aim of the work reported in this paper was mainly to determine whether H a r p e r's observations on the uneven distribution of fungistatic bacteria in the rhizosphere of different banana varieties as related with the resistance to Panama Disease, had a general validity. Unfortunately our investigations did not yield any support for this possibility.

The banana rhizosphere always favoured the development of the group of micro-organisms referred to in the tables as "others". This is in agreement with what L o c h h e a d [2]) found as a general rule: that the rhizosphere population always supports a higher proportion of coloured and motile organisms. (Tables I, IV, and V).

Neither large numbers of fungistatic micro-organisms nor an uneven distribution between the two banana varieties used in the experiments was found to indicate that both or one of them yielded in watery rhizome extracts substances which would either enhance or discourage the formation of fungistatic antibiotics. Nor was there any indication that under the influence of rhizome extracts the Panama Disease fungus became specifically susceptible or resistant against any compound produced by the rhizosphere organisms (Table III).

The rate of decline of *Fusarium oxysporum-cubense* in flowerpots filled with soil which had been exposed previously in the field to pro-

TABLE IX

Distribution of fungistatic micro-organisms in the rhizosphere of various banana varieties

Clone	Symptoms of P.D.	Number of colonies tested	Number of active	
			bacteria	Actyno-mycetes
Field B				
G.M. 1/2	No	75	2	0
G.M. 1/4	No	63	0	0
G.M. 1/6	Yes(?)	91	0	2
G.M. 2/1	No	62	0	0
G.M. 2/4	No	108	1	1
G.M. 2/6	Yes	103	3	0
G.M. 3/1	No	56	0	0
G.M. 3/2	No	94	1	2
G.M. 3/3	No	63	0	0
G.M. 3/4	No	95	0	0
G.M. 4/1	Yes(?)	107	0	0
G.M. 4/3	Yes(?)	100	0	4
G.M. 5/1	Yes(?)	59	0	2
G.M. 5/3	Yes	51	0	1
G.M. 6/2	Yes	45	0	0
G.M. 6/3	Yes(?)	96	2	2
G.M. 7/1	Yes(?)	75	0	3
G.M. 7/3	No	103	2	0
G.M. 7/6	Yes	105	3	0
G.M. 8/5	Yes	54	0	0
Congo 1	No	60	0	0
Congo 2	No	87	1	0
Congo 3	No	63	0	0
Dichotomous Chinese 1	No	106	1	1
Dichotomous Chinese 3	No	80	0	0
Dwarf Chinese 3	No	98	0	2
Extra dwarf Chinese 2	No	94	2	0
Masak hijau 1	No	98	2	0
Masak hijau 2	No	93	2	2
Masak hijau 3	No	95	2	2
Field 14A				
Congo 1/3	No	58	0	1
Congo 2/1	No	85	0	0
Congo 3/4	No	74	1	3
Hamakua 8/3	No	110	2	2
Lacatan 7/5	No	89	1	2
Manaiula 2/4	No	55	0	0
Manaiula 3/2	No	78	0	1
Manaiula 4/3	No	84	0	0
Masak hijau 4/4	No	90	2	2
Masak hijau 5/5	No	81	2	1

ducts excreted by or otherwise derived from the roots of the two banana varieties, was virtually identical. A calculated regression of the number of viable isolates of *F. oxysporum-cubense* on time from inoculation gives a regression coefficient $b = -38.0$ for Gros Michel and $b = -37.6$ for Congo. These are not significantly different.

The contrast between the results obtained by Harper and the outcome of this investigation is difficult to explain. Harper collected his roots from a field which was shortly afterwards completely occupied by newly erected buildings. It was, therefore, not possible to re-examine banana plants grown at the same site. It is conceivable that peculiar local conditions of drainage, fertilisation, weed flora, etc., may have been responsible for the facts he observed.

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SUMMARY

1. Observations on the composition of the micro-flora in the rhizosphere of Gros Michel and Congo banana are communicated. Bacteria, other than aerobic spore bearing species, are preferentially supported by the roots of both varieties.

2. Micro-organisms capable of producing antibiotics inhibiting the growth of *Fusarium oxysporum-cubense* were occasionally met with but there was no difference in their distribution over the two banana varieties.

3. No specific influence of soil types, showing extreme different grades of severeness of Panama Disease, on the composition of the rhizosphere microflora could be observed.

4. No specific influence of roots of the two banana varieties on the survival time of *Fusarium oxysporum-cubense* in soil samples could be detected.

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